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(54) Title: NEW BIOLOGICAL ENTITIES AND THE TARGETED USE THEREOF

(57) Abstract: The present invention provides method for the treatment of a disease by applying a medicament comprising a protease with a defined specificity is capable to hydrolyze specific peptide bonds within a target substrate related to such disease. The proteases with such a defined specificity can further be used for related therapeutic or diagnostic purposes.

New Biological Entities and the Targeted Use Thereof

The present invention provides methods for the treatment of a disease by applying a medicament comprising a protease with a defined specificity is capable to hydrolyze specific peptide bonds within a target substrate related to such disease. The proteases with such a defined specificity can further be used for related therapeutic or diagnostic purposes.

Background

Academic and industrial research continuously searches for functional proteins to be used as therapeutic, research, diagnostic, nutritional, personal care or industrial agents. Today, such functional proteins can be classified mainly into two categories: natural proteins and engineered proteins. Natural proteins, on the one hand, are discovered from nature, e.g. by screening natural isolates or by sequencing genomes from diverse species. Engineered proteins, on the other hand, are typically based on known proteins and are altered in order to acquire modified functionalities. The present invention discloses engineered proteins with novel functions as compared to the starting components. Such proteins are called NBEs (New Biologic Entities). The NBEs disclosed in the present invention are engineered enzymes with novel substrate specificities or fusion proteins of such engineered enzymes with other functional components.

Specificity is an essential element of enzyme function. A cell consists of thousands of different, highly reactive catalysts. Yet the cell is able to maintain a coordinated metabolism and a highly organized three-dimensional structure. This is due in part to the specificity of enzymes, i.e. the selective conversion of their respective substrates. Specificity is a qualitative and a quantitative property: the specificity of a particular enzyme can vary widely, ranging from just one particular type of target molecules to all molecular types with certain chemical substructures. In nature, the specificity of an organism's enzymes has been evolved to the particular needs of the organism. Arbitrary specificities with high value for therapeutic, research, diagnostic, nutritional or industrial applications are unlikely to be found in any organism's enzymatic repertoire due to the large space of possible specificities. The only realistic way of obtaining such specificities is their generation de novo.

When comparing enzymes with binders, a paradigm of specificity is given by antibodies recognizing individual epitopes as small distinct structures within large molecules. The naturally occurring vast range of antibody specificities is attributed to the diversity generated by the immune system combined with natural selection. Several mechanisms contribute to the vast repertoire of antibody specificity and occur at different stages of immune response generation and antibody maturation (Janeway, C et al. (1999) Immunobiology, Elsevier Science Ltd., Garland Publishing, New York). Specifically, antibodies contain complementarity determining regions (CDRs) which interact with the antigen in a highly specific manner and allow discrimination even between very similar epitopes. The light as well as the heavy chain of the antibody each contribute three CDRs to the binding domain. Nature uses recombination of various gene segments combined with further mutagenesis in the generation of CDRs. As a result, the sequences of the six CDR loops are highly variable in composition and length and this forms the basis for the diversity of binding specificities in antibodies. A similar principle for the generation of a diversity of catalytic specificities is not known from nature.

Catalysis, i.e. the increase of the rate of a specific chemical reaction, is besides binding the most important protein function. Catalytic proteins, i.e. enzymes, are classified according to the chemical reaction they catalyze.

Transferases are enzymes transferring a group, for example, the methyl group or a glycosyl group, from one compound (generally regarded as donor) to another compound (generally regarded as acceptor). For example, glycosyltransferases (EC 2.4) transfer glycosyl residues from a donor to an acceptor molecule. Some of the glycosyltransferases also catalyze hydrolysis, which can be regarded as transfer of a glycosyl group from the donor to water. The subclass is further subdivided into hexosyltransferases (EC 2.4.1), pentosyltransferases (EC 2.4.2) and those transferring other glycosyl groups (EC 2.4.99, Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB)).

Oxidoreductases catalyze oxido-reductions. The substrate that is oxidized is regarded as hydrogen or electron donor. Oxidoreductases are classified as dehydrogenases, oxidases, mono- and dioxygenases. Dehydrogenases transfer hydrogen from a hydrogen donor to a hydrogen acceptor molecule. Oxidases react with molecular oxygen as hydrogen acceptor and produce oxidized products as well as either hydrogen peroxide or water. Monooxygenases transfer one oxygen atom from molecular oxygen to the substrate and one is reduced to water. In contrast,

dioxygenases catalyze the insert of both oxygen atoms from molecular oxygen into the substrate.

Lyases calalyze elimination reactions and thereby generate double bonds or, in the reverse direction, catalyze the additions at double bonds. Isomerases catalyze intramolecular rearrangements. Ligases catalyze the formation of chemical bonds at the expense of ATP consumption.

Finally, hydrolases are enzymes that catalyze the hydrolysis of chemical bonds like C-O or C-N. The E.C. classification for these enzymes generally classifies them by the nature of the bond hydrolysed and by the nature of the substrate. Hydrolases such as lipases and proteases play an important role in nature as well in technical applications of biocatalysts. Proteases hydrolyse a peptide bond within the context of an oligo- or polypeptide. Depending on the catalytic mechanism proteases are grouped into aspartic, serin, cysteine, metallo- and threonine proteases (Handbook of proteolytic enzymes. (1998) Eds: Barret, A; Rawling, N.; Woessner, J.; Academic Press, London). This classification is based on the amino acid side chains that are responsible for catalysis and which are typically presented in the active site in very similar orientation to each other. The scissile bond of the substrate is brought into register with the catalytic residues due to specific interactions between the amino acid side chains of the substrate and complementary regions of the protease (Perona, J. & Craik, C (1995) *Protein Science*, 4, 337-360). The residues on the N- and C-terminal side of the scissile bond are usually called P_1, P_2, P_3 etc and P_1', P_2', P_3' and the binding pockets complementary to the substrate S_1, S_2, S_3 and S_1', S_2', S_3', respectively (nomenclature according to Schlechter & Berger, Biochem. Biophys. Res. Commun. 27 (1967) 157-162). The selectivity of proteases can vary widely from being virtually nonselective – e.g. the Subtilisins – over a strict preference at the R position – e.g. Trypsin selectively cutting on the C-terminal side of arginine or lysine residues – to highly specific proteases – e.g. human tissue-type plasminogen activator (t-PA) cleaving at the C-terminal side of the arginine in the sequence CPGRVVG (Ding, L et al. (1995) Proc. Natl. Acad. Sci. USA 92, 7627-7631; Coombs, G et al. (1996) *J. Biol. Chem*. 271, 4461-4467).

The specificity of proteases, i.e. their ability to recognize and hydrolyze preferentially certain peptide substrates, can be expressed qualitatively and quantitatively. Qualitative specificity refers to the kind of amino acid residues that are accepted by a protease at certain positions of the peptide substrate. For example, trypsin and t-PA are related with respect to their qualitative specificity,

since both of them require at the P_1 position an arginine or a similar residue. On the other hand, quantitative specificity refers to the relative number of peptide substrates that are accepted as substrates by the protease, or more precisely, to the relative k_{cat}/k_M ratios of the protease for the different peptides that are accepted by the protease. Proteases that accept only a small portion of all possible peptides have a high specificity, whereas the specificity of proteases that, as an extreme, cleave any peptide substrate would theoretically be zero.

Comparison of the primary, secondary as well as the tertiary structure of proteases (Fersht, A., Enzyme Structure and Mechanism, W. H. Freeman and Company, New York, 1995) allows identification of classes showing a high degree of conservation (Rawlings, N.D. & Barrett, A.J. (1997) In: *Proteolysis in Cell Functions* Eds. Hopsu-Havu,V.K.; Järvinen,M.; Kirschke,H, pp. 13-21, IOS Press, Amsterdam). A widely accepted scheme for protease classification has been proposed by Rawlings & Barrett (Handbook of proteolytic enzymes. (1998) Eds: Barret, A; Rawling, N.; Woessner, J.; Academic Press, London). For example, the serine proteases family can be subdivided into structural classes with chymotrypsin (class S1), subtilisin (class S8) and carboxypeptidase (class SC) folds, each of which includes nonspecific as well as specific proteases (Rawlings, N.D. & Barrett, A.J. (1994) *Methods Enzymol.* 244, 19-61). This applies to other protease families analogously. An additional distinction can be made according to the relative location of the cleaved bond in the substrate. Carboxy- and aminopeptidases cleave amino acids from the C- and N-terminus, respectively, while endopeptidases cut anywhere along the oligopeptide.

Many applications would be conceivable if enzymes with a basically unlimited spectrum of specificities were available. However, the use of such enzymes with high, low or any defined specificity is currently limited to those which can be isolated from natural sources. The field of application for these enzymes varies from therapeutic, research, diagnostic, nutritional to personal care and industrial purposes.

Enzyme additives in detergents have come to constitute nearly a third of the whole industrial enzyme market. Detergent enzymes include proteinases for removing organic stains, lipases for removing greasy stains, amylases for removing residues of starchy foods and cellulases for restoring of smooth surface of the fiber. The best-known detergent enzyme is probably the nonspecific proteinase subtilisin, isolated from various *Bacillus* species.

Starch enzymes, such as amylases, occupy the majority of those used in food processing. While starch enzymes include products that are important for textile desizing, alcohol fermentation, paper and pulp processing, and laundry detergent additives, the largest application is for the production of high fructose corn syrup. The production of corn syrup from starch by means of industrial enzymes was a successful alternative to acid hydrolysis.

Apart from starch processing, enzymes are used for an increasing range of applications in food. Enzymes in food can improve texture, appearance and nutritional value or may generate desirable flavours and aromas. Currently used food enzymes in bakery are amylase, amyloglycosidases, pentosanases for breakdown of pentosan and reduced gluten production or glucose oxidases to increase the stability of dough. Common enzymes for dairy are rennet (protease) as coagulant in cheese production, lactase for hydrolysis of lactose, protease for hydrolysis of whey proteins or catalase for the removel of hydrogen peroxides. Enzymes used in brewing process are the above named amylases, but also cellulases or proteases to clarify the beer from suspended proteins. In wines and fruit juices, cloudiness is more commenly caused by starch and pectins so that amylases and pectinases increase yield and clarification. Papain and other proteinases are used for meat tenderizing.

Enzymes have also been developed to aid animals in the digestion of feed. In the western hemisphere, corn is a major source of food for cattle, swine, and poultry. In order to improve the bioavailability of phosphate from corn, phytase is commonly added (Wyss, M. et al., Biochemical characterization of fungal phytases (myo-inositol hexakisphosphate phosphohydrolases); Catalytic properties. Applied & Environmental Microbiology 65, 367-373 (1999)). Moreover, phytate hydrolysis has been shown to bring about improvements in digestibility of protein and absorption of minerals such as calcium (Bedford, M.R. & Schulze, H., Exogenous Enzymes for Pigs and Poultry [Review]. Nutrition Research Reviews 11, 91-114 (1998)). Another major feed enzyme is xylanase. This enzyme is particularly useful as a supplement for feeding stuff comprising more than about 10% of wheat barley or rye, because of their relatively high soluble fiber content. Xylanases cause two important actions: reduction of viscosity of the intestinal contents by hydrolyzing the gel-like high molecular weight arabinoxylans in feed (Murphy, T et al., Effect of range of new xylanases on in vitro viscosity and on performance of broiler diets.

British Pultry Science 44, S16-S18 (2003)) and break down of polymers in cell wallswhich improve the bioavailability of protein and starch.

Biotech research and development laboratories routinely use special enzymes in small quantities along with many other reagents. These enzymes create a significant market for various enzymes. Enzymes like alkaline phosphatase, horseradish peroxidase and luciferase are only some examples. Thermostable DNA polymerases like Taq polymerase or restriction endonucleases revolutionized laboratory work.

The use of enzymes in the diagnosis of disease is another important benefit derived from the intensive research in biochemistry. Within the recent past few years that interest in diagnostic enzymology has increased and there are still large areas of medical research in which the diagnostic potential of enzyme reactions has not been explored at all. Common enzymes used for clinical diagnosis are acid phosphatase, alanine aminotransferase, alkaline phosphatase, amylase, angiotensin converting enzymes, aspartate aminotransferase, cholinesterase, creatinine kinase, gamma glutamyltransferase, lactate dehydrogenaseor rennin.

Therapeutic enzymes are a particular class of drugs, categorized by the FDA as biologicals, with a lot of advantages compared to other, especially non-biological pharmaceuticals. Examples for successful therapeutic enzymes are human clotting factors like factor VIII and factor IX for human treatment. In addition, digestive enzymes are used for various deficiencies in human digestive processes. Other examples are t-PA and streptokinase for the treatment of cardiovascular disease, beta-glucocerebrosidase for the treatment of Type I Gaucher disease, L-asparaginase for the the treatment of acute lymphoblastic leukemia and DNAse for the treatment of cystic fibrosis. An important issue in the application of proteins as therapeutics is their potential immunogenicity. To reduce this risk, one would prefer enzymes of human origin, which narrows down the set of available enzymes. The provision of designed enzymes, preferably of human origin, with novel, tailor-made specificities would allow the specific modification of target substrates at will, while minimizing the risk of immunogenicity. A further advantage of highly specific enzymes as therapeutics would be their lower risk of side effects. Due to the limited possibility of specific interactions between a small molecule and a protein, binding to non-target proteins and therefore side effects are quite common and often cause termination of an otherwise promising lead compound. Specific enzymes, on the other hand, provide many more contact sites and mechanisms for substrate

discrimination and therefore enable a higher specificity and thereby less side activities.

Proteases represent an important class of therapeutic agents (*Drugs of today*, 33, 641-648 (1997)). However, currently the therapeutic protease is usually a substitute for insufficient acitivity of the body's own proteases. For example, factor VII can be administered in certain cases of coagulation deficiencies of bleeders or during surgery (Heuer L.; Blumenberg D. (2002) *Anaesthesist* 51:388). Tissue-type plasminogen activator (t-PA) is applied in acute cardiac infarction, initializing the dissolution of fibrin clots through specific cleavage and activation of plasminogen (Verstraete, M. et al. (1995) *Drugs*, 50, 29-41). So far a protease with taylor-made specificity is generated to provide a therapeutic agent that specifically activates or inactivates a disease related target protein.

Monoclonal antibodies represent another important biological class of substances with therapeutic capabilities. One of the main antibody targets are tumor necrosis factors (TNFs) which belong to the family of cytokines. TNFs play a major role in the inflammation process. As homotrimers they could bind to receptors of nearly every cell. They activate a multiplicity of cellular genes, multiple signal transduction mechanisms, kinases and transcription factors. The most important TNFs are TNF-alpha and TNF-beta. TNF-alpha is produced by macrophages, monocytes and other cells. TNF-alpha is an inflammation mediator. Therefore, research of the last decade has been focused on TNF-alpha inhibitors like monoclonal antibodies as possible therapeutics for different therapeutic indications like Rheumatoid Arthritis, Crohn´s disease or Psoriasis (Hamilton et al. (2000) Expert Opin Pharmacother, 1 (5): 1041-1052). One of the major disadvantages of monoclonal antibodies are their high costs, so that new biological alternatives are of great importance.

There are a lot of examples for engineered enzymes in literature. Fulani et al. (Fulani F. et al. (2003) *Protein Engineering* 16, 515-519) describe a rhodanase (thiosulfat:cyanide sulfurtransferase) from Azotobacter vinelandii which has a catalytic domain structurally related to catalytic subunit of Cdc25 phosphatase enzymes. The difference in catalytic mechanism depends on the different size of the active site. Both rhodanase and phosphatase are highly specific on different substrates (sulfate vs. phosphate). The catalytic mechanism of the rhodanase could be shifted towards serine/threonine phosphatase by single-residue insertion. Therefore, Fulani et al. give a single example for the change of a catalytic mechanism by structural comparison and sequence alignment of naturally known

enzymes from different enzyme classes but lack an indication of how to generate a user-definable substrate specificity while keeping the same catalytic mechanism.

The thioredoxin reductase described by Briggs et al. (WO 02/090300 A2) has an altered cofactor specificity which preferably binds NADPH compared to NADH. Thus, both enzymes, the starting point as well as the resulting engineered enzyme are highly specific towards different substrates. The methods to achieve such an altered substrate specificity are either computational processing methods or sequence alignments of related proteins to define variable and conserved residues. They all have in common that they are based on the comparison of structures and sequences of proteins with known specificities followed by the transfer of the same to another backbone.

There are other examples of specificity-engineered enzymes and, in particular, of proteases which have been published in the literature. None of these examples, however, provides a means for generating novel specificites compared to the specificity of the starting material used within the described methods. The methods range from structure-directed single point mutations (Kurth, T. et al. (1998) *Biochemistry* 37, 11434-11440; Ballinger, M et al. (1996) *Biochemistry*, 35:13579-13585), exchange of surface loops between two specific proteases (Horrevoets et al. (1993) *J. Biol. Chem.* 268, 779-782), to random mutagenesis either regio-selectively or across the whole gene combined with in-vitro or in-vivo selection (Sices, H. & Kristie, T. (1998) *Proc. Natl. Acad. Sci. USA*, 95, 2828-2833).

The rational design of protease specificity is limited to very few examples. This approach is severely limited by the insufficient understanding of the complexities that govern folding and dynamics as well as structure-function relationships in proteins (Corey, M.J. & Corey, E. (1996) *Proc. Natl. Acad. Sci. USA*, 93:11428-11434). It is therefore difficult to alter the primary amino acid sequence of a protease in order to change its activity or specificity in a predictive way. In a successful example, Kurth et al. engineered trypsin to show a preference for a dibasic motive (Kurth, T. et al. (1998) *Biochemistry*, 37:11434-11440). In another example, Hedstrom et al. converted the S_1 substrate specificity of trypsin to that of chymotrypsin (Hedstrom, L. et al. (1992) Science, 255:1249-1253). This is an example where a known property was transferred from one backbone to another.

Ballinger et al. (WO 96/27671) describe subtilisin variants with combination mutations (N62D/G166D, and optionally Y104D) having a shift of substrate

specificity towards peptide or polypeptide substrates with basic amino acids at the P1, P2 and P4 positions of the substrate. Suitable substrates of the variant subtilisin were revealed by sorting a library of phage particles (substrate phage) containing five contiguous randomized residues. These subtilisin variants are useful for cleaving fusion proteins with basic substrate linkers and processing hormones or other proteins (in vitro or in vivo) that contain basic cleavage sites.

The problems associated with rational redesign of enzymes can partially be overcome by directed evolution (as disclosed in PCT/EP03/04864). These studies can be classified by their expression and selection systems. Genetic selection means to produce inside an organism an enzyme, e.g. a protease, which is able to cleave a precursor protein which in turn results in an alteration of the growth behavior of the producing organism. From a population of organisms with different proteases those can be selected which have an altered growth behavior. This principle was for example reported by Davis et al. (US 5258289, WO 96/21009). The production of a phage system is dependent on the cleavage of a phage protein which only can be activated in the presence of a proteolytic enzyme which is able to cleave the phage protein. Other approaches use a reporter system which allows a selection by screening instead of a genetic selection, but also cannot overcome the intrinsic insufficiency of the intracellular characterization of enzymes.

Systems to generate enzymes with altered sequence specificities with self-secreting enzymes are also reported. Duff et al. (WO 98/11237) describe an expression system for a self-secreting protease. An essential element of the experimental design is that the catalytic reaction acts on the protease itself by an autoproteolytic processing of the membrane-bound precursor molecule to release the matured protease from the cellular membrane into the extracellular environment. Therefore, a fusion protein must be constructed where the target peptide sequence replaces the natural cleavage site for autoproteolysis. Limitations of such a system are that positively identified proteases will have the ability to cleave a certain amino acid sequence but they also may cleave many other peptide sequences. Therefore, high substrate specificity cannot be achieved. Additionally, such a system is not able to control that selected proteases cleave at a specific position in a defined amino acid sequence and it does not allow a precise characterization of the kinetic constants of the selected proteases (k_{cat}, K_M).

A method has been described that aims at the generation of new catalytic activities and specificities within the α/ß-barrel proteins (WO 01/42432; Fersht et al, Methods

of producing novel enzymes; Altamirano et al. (2000) *Nature* 403, 617-622). The α/ß-barrel proteins comprise a large superfamily of proteins accounting for a large fraction of all known enzymes. The structure of the proteins is made from a/ß-barrel surrounded by α-helices. The loops connecting ß-strands and helices comprise the so-called lid-structure including the acitve site residues. The method is based on the classification of α/ß-barrel proteins into two classes based on the catalytic lid structure. An extensive comparison of α/ß-barrel protein structures led the authors to the conclusion that the substrate binding and specificity is primarily defined by the barrel structure while the specificity of the chemical reaction resides within the loops. It is suggested that barrels and lid structures from different enzymes can be combined to generate new enzymatic activities and to provide a starting point to fine tune the properties by targeted or randomized mutagenesis and selection. The method does not provide for the generation of user-defined specificity.

In summary, it is clear that there are many possible applications in the fields of therapeutics, research and diagnostics, industrial enzymes, food and feed processing, cosmetics and other areas that would become possible by the availability of enzymes with a novel substrate specificity. However, only a limited number of specific enzymes has been identified from natural sources so far. Methods of rational design to modify, alter, convert or transfer sequence specificity as well as random approaches described above did not enable the generation of a novel and user-definablespecificity that was not present in the employed starting material.

Therefore, none of the currently available methods can provide enzymes with a novel and user-defined sequence specificity. Such enzymes were disclosed in applicant's yet unpublished applications PCT/EP2004/051172, PCT/EP2004/051173; USSN 10/872,197 and USSN 10/872,198. The current invention provides further such enzymes as well as methods for generating them.

Summary of the Invention

The objective of the present invention is to provide a method for the treatment of a disease by applying a medicament comprising a protease. Further the present invention provides engineered proteins with novel functions that do not exist in the components used for the engineering of such proteins. In particular, the invention provides enzymes with user-definable specificities. User-definable specificity means

that enzymes are provided with specificities that do not exist in the components used for the engineering of such enzymes. The specificities can be chosen by the user so that one or more intended target substrates are preferentially recognised and converted by the enzymes. Furthermore, the invention provides enzymes that possess essentially identical sequences to human proteins but have different specificities. In a particular embodiment, the invention provides proteases with user-definable specificities.

Furthermore, the present invention is directed to engineered enzymes which are fused to one or more further functional components. These further components can be proteinacious components which preferably have binding properties and are of the group consisting of substrate binding domains, antibodies, receptors or fragments thereof. In a particular aspect, the engineered proteases are fused to proteins or peptide sequences that bind to marker molecules that are only present or over-expressed in specific tissues, specific organs, specific cell types, specific diseases or a combination thereof, thereby increasing the half-life of the engineered proteases or increasing the local concentration in the respective tissues, organs or diseased areas of the body. In another aspect the engineered proteases are fused to proteins or peptide sequences that bind to the target molecule of the engineered protease, thereby increasing the interaction between protease and target. In another aspect of the invention the engineered proteases are fused to proteins or peptide sequences that reduce the rate of clearance from the serum after i.v. administration. In another aspect of the invention the engineered proteases are fused to proteins or peptide sequences that trigger the import of the protease into target cells or the transport of the proteases across the blood brain barrier.

Furthermore, the above further components can be further functional components, preferably being selected from the group consisting of polyethylenglycols, carbohydrates, lipids, fatty acids, nucleic acids, metals, metal chelates, and fragments or derivatives thereof. The resulting fusion proteins are understood as enzymes with user-definable specificities within the present invention.

Besides, the invention is directed to the application of such enzymes with novel, user-definable specificities for therapeutic, research, diagnostic, nutritional, personal care or industrial purposes. Moreover, the invention is directed to a method for generating engineered enzymes with user-definable specificities. In particular, the invention is directed to generate enzymes that possess essentially identical sequences to human enzymes but have different specificities.

This problem has been solved by the embodiments of the invention specified in the description below and in the claims. The present invention is thus directed to

(1) the use of a protease with defined specificity for a target substrate for preparing a medicament for the treatment of a specific disease related to said target substrate,

(2) an engineered enzyme with defined specificity characterized by the combination of the following components,:
(a) a protein scaffold which catalyzes at least one chemical reaction on at least one substrate, and
(b) one or more specificity determining regions (SDRs) located at sites in the protein scaffold that enable the resulting engineered protein to discriminate between at least one target substrate and one or more different substrates, and herein the SDRs are essentially synthetic peptide sequences;

(3) the use of an engineered enzyme as defined in (2) above for therapeutic, research, diagnostic, nutritional, personal care or industrial purposes, preferably for the use as defined in (1) above;

(4) a method for generating engineered enzymes as defined in (2) above having specificities towards target substrates, such specificities not being present in the individual starting components, comprising at least the following steps:
(a) providing a protein scaffold which catalyzes at least one chemical reaction on at least one substrate,
(b) generating a library of engineered enzymes by combining the protein scaffold from step (a) with fully or partially random peptide sequences at sites in the protein scaffold that enable the resulting engineered enzyme to discriminate between at least one target substrate and one or more different substrates, and
(c) selecting out of the library of engineered enzymes generated in step (b) one or more enzymes that have specificities towards at least one target substrate;

(5) a fusion protein which is comprised of at least one engineered enzyme as defined in (2) above and at least one further component, preferably the at least one further component having binding properties and more preferably being selected from the group consisting of antibodies, binding domains, receptors, and fragments thereof;

(6) a composition or pharmaceutical composition comprising one or more engineered enzymes as defined in (2) above or a fusion protein as defined in (5)

above, said pharmaceutical composition may optionally comprise an acceptable carrier, excipient and/or auxiliary agent;

(7) a DNA encoding the engineered enzyme as defined in (2) above;

(8) a vector comprising the DNA as defined in (7) above;

(9) a host cell or transgenic organism being transformed/transfected with a vector as defined in (8) above and/or containing the DNA as defined in (7) above; and

(10) a method for producing the engineered enzyme of (2) above comprising culturing a cell or organism as defined in (8) above and isolating the enzyme from the culture broth.

Brief description of the Figures

The following figures are provided in order to explain further the present invention in supplement to the detailed description:

Figure 1 illustrates the three-dimensional structure of human trypsin I with the active site residues shown in "ball-and-stick" representation and with the marked regions indicating potential SDR insertion sites.

Figure 2 shows the alignment of the primary amino acid sequence of three members of the serine protease class S1 family: human trypsin I, human alpha-thrombin and human enteropeptidase (see also SEQ ID NOs: 1, 5 and 6).

Figure 3 illustrates the three-dimensional structure of subtilisin with the active site residues being shown in "ball-and-stick" representation and with the numbered regions indicating potential SDR insertion sites.

Figure 4 shows the alignment of the primary amino acid sequences of four members of the serine protease class S8 family: subtilisin E, furin, PC1 and PC5 (see also SEQ ID NOs: 7-10).

Figure 5 illustrates the three-dimensional structure of pepsin with the active site residues being shown in "ball-and-stick" representation and with the numbered regions indicating potential SDR insertion sites.

Figure 6 shows the alignment of the primary amino acid sequences of three members of the A1 aspartic acid protease family: pepsin, ß-secretase and cathepsin D (see also SEQ ID NOs: 11-13).

Figure 7: illustrates the three-dimensional structure of caspase 7 with the active site residues being shown in "ball-and-stick" representation and with the numbered regions indicating potential SDR insertion sites.

Figure 8: shows the primary amino acid sequence of caspase 7 as a member of the cysteine protease class C14 family (see also SEQ ID NO: 14).

Figure 9 depicts schematically the third aspect of the invention.

Figure 10 shows a Western blot analysis of a culture supernatant of cells expressing variants of human trypsin I with SDR1 and SDR2, compared to negative controls.

Figure 11 shows the time course of the proteolytic cleavage of a target substrate by human trypsin I.

Figure 12 shows the relative activities of three variants of inventive engineered proteolytic enzymes in comparison with human trypsin I on two different peptide substrates.

Figure 13 shows the relative specificities of human trypsin I and variants of inventive engineered proteolytic enzymes with one or two SDRs, respectively.

Figure 14: shows the relative specificities of human trypsin I and of variants of inventive engineered proteolytic enzymes being specific for human TNF-alpha with this scaffold on peptides with a target sequence of human TNF-alpha.

Figure 15: shows the reduction of cytotoxicity induced by TNF-alpha when incubating the TNF-alpha with concentrated supernatant from cultures expressing the inventive engineered proteolytic enzymes being specific for human TNF-alpha.

Figure 16: shows the reduction of cytotoxicity induced by TNF-alpha when incubating the TNF-alpha with purified inventive engineered proteolytic enzyme being specific for human TNF-alpha.

Figure 17: compares the activity of inventive engineered proteolytic enzymes being specific for human TNF-alpha with the activity of human trypsin I on two protein substrates: (a) human TNF-alpha; (b) mixture of human serum proteins.

Figure 18: showes the specific activity of an inventive engineered proteolytic enzyme with specificity for human VEGF.

Definitions

In the framework of the present invention the following terms and definitions are used.

The term "protease" means any protein molecule that is capable of hydrolysing peptide bonds. This includes naturally-occurring or artificial proteolytic enzymes, as well as variants thereof obtained by site-directed or random mutagenesis or any other protein engineering method, any active fragment of a proteolytic enzyme, or any molecular complex or fusion protein comprising one of the aforementioned proteins. A "chimera of proteases" means a fusion protein of two or more fragments derived from different parent proteases.

The term "substrate" means any molecule that can be converted catalytically by an enzyme. The term "peptide substrate" means any peptide, oligopeptide, or protein molecule of any amino acid composition, sequence or length, that contains a peptide bond that can be hydrolyzed catalytically by a protease. The peptide bond that is hydrolyzed is referred to as the "cleavage site". Numbering of positions in the substrate is done according to the system introduced by Schlechter & Berger (Biochem. Biophys. Res. Commun. 27 (1967) 157-162). Amino acid residues adjacent N-terminal to the cleavage site are numbered P_1, P_2, P_3, etc., whereas residues adjacent C-terminal to the cleavage site are numbered P_1', P_2', P_3', etc.

The term "target substrate" describes a user-defined substrate which is specifically recognized and converted by an enzyme according to the invention. The term "target peptide substrate" describes a user-defined peptide substrate. The term "target specificity" describes the qualitative and quantitative specificity of an enzyme that is capable of recognizing and converting a target substrate.

Catalytic properties of enzymes are expressed using the kinetic parameters "K_M" or "Michaelis Menten constant", "k_{cat}" or "catalytic rate constant", and "k_{cat}/K_M" or "catalytic efficiency", according to the definitions of Michaelis and Menten (Fersht, A., Enzyme Structure and Mechanism, W. H. Freeman and Company, New York, 1995). The term "catalytic activity" describes quantitatively the conversion of a given substrate under defined reaction conditions.

The term "specificity" means the ability of an enzyme to recognize and convert preferentially certain substrates. Specificity can be expressed qualitatively and quantitatively. "Qualitative specificity" refers to the chemical nature of the substrate residues that are recognized by an enzyme. "Quantitative specificity" refers to the number of substrates that are accepted as substrates. Quantitative specificity can be expressed by the term s, which is defined as the negative logarithm of the number of all accepted substrates divided by the number of all

possible substrates. Proteases, for example, that accept preferantially a small portion of all possible peptide substrates have a "high specificity". Proteases that accept almost any peptide substrate have a "low specificity". Definitions are made in accordance to WO 03/095670 which is therefore incorporated by reference.Proteases with very low specificity are also referred to as "unspecific proteases". The term "defined specificity" refers to a certain type of specificity, i.e. to a certain target subtrate or a set of certain target substrates that are preferentially converted versus other substrates.

The term "engineered" in combination with the term "enzyme" describes an enzyme that is comprised of different components and that has features not being conferred by the individual components alone.

The term "protein scaffold" or "scaffold protein" refers to a variety of primary, secondary and tertiary polypeptide structures.

The term "peptide sequence" indicates any peptide sequence used for insertion or substitution into or combination with a protein scaffold. Peptide sequences are usually obtained by expression from DNA sequences which can be synthesized according to well-established techniques or can be obtained from natural sources. Insertion, substitution or combination of peptide sequences with the protein scaffold are generated by insertion, substitution or combination of oligonucleotides into or with a polynucleotide encoding the protein scaffold. The term "synthetic" in combination with the term "peptide sequence" refers to peptide sequences that are not present in the protein scaffold in which the peptide sequences are inserted or substituted or with which they are combined.

The term "components" in combination with the term "engineered enzyme" refers to peptide or polypeptide sequences that are combined in the engineering of such enzymes. Such components may among others comprise one or more protein scaffolds and one or more synthetic peptide sequences. The term "library of engineered enzymes" describes a mixture of engineered enzymes, whereby every single engineered enzyme is encoded by a different polynucleotide sequence. The term "gene library" indicates a library of polynucleotides that encodes the library of engineered enzymes. The term "SDR" or "Specificity determining region" refers to a synthetic peptide sequence that provides the defined specificity when combined with the protein scaffold at sites that enable the resulting enzymes to discriminate

between the target substrate and one or more other substrates. Such sites are termed "SDR sites".

The terms "tertiary structure similar to the structure of" and "similar tertiary structure" in combination with the terms "enzyme" or "protein" refer to proteins in which the type, sequence, connectivity and relative orientation of the typical secondary structural elements of a protein, e.g. alpha-helices, beta-sheets, beta-turns and loops, are similar and the proteins are therefore grouped into the same structural or topological class or fold. This includes proteins that have altered, additional or deleted structural elements of any type but otherwise unchanged topology. Examples of such structural classes are the TNF superfamily, the S1 fold or the S8 fold within the serine proteases, the GPCRs, or the α/ß-barrel fold.

The term "positions that correspond structurally" indicates amino acids in proteins of similar tertiary structure that correspond structurally to each other, i.e. they are usually located within the same structural or topological element of the structure. Within the structural element they possess the same relative positions with respect to beginning and end of the structural element. If, e.g. the topological comparison of two proteins reveals two structurally corresponding sequences of different length, then amino acids within, e.g. 20% and 40% of the respective region lengths, correspond to each other structurally.

The term "library of engineered enzymes" of the present invention refers to a multiplicity of enzymes or enzyme variants, which may exist as a mixture or in isolated form.

Amino acids residues are abbreviated according to the following Table 1 either in one- or in three-letter code.

Table 1: Amino acid abbreviations

Abbreviations		Amino acid	Abbreviations		Amino acid
A	Ala	Alanine	M	Met	Methionine
C	Cys	Cysteine	N	Asn	Asparagine
D	Asp	Aspartic acid	P	Pro	Proline
E	Glu	Glutamic acid	Q	Gln	Glutamine
F	Phe	Phenylalanine	R	Arg	Arginine
G	Gly	Glycine	S	Ser	Serine
H	His	Histidine	T	Thr	Threonine

I	Ile	Isoleucine	V	Val	Valine
K	Lys	Lysine	W	Trp	Tryptophane
L	Leu	Leucine	Y	Tyr	Tyrosine

Detailed description of the invention

The present invention provides engineered proteins with novel functions. In particular, the invention provides enzymes with user-definable specificities. In a particular embodiment, the invention provides proteases with user-definable specificities. Besides, the invention provides applications of such enzymes with novel, user-definable specificities for therapeutic, research, diagnostic, nutritional, personal care or industrial purposes. Moreover, the invention provides a method for generating enzymes with specificities that are not present in the components used for the engineering of such enzymes. In particular, the invention is directed to the generation of enzymes that have sequences that are essentially identical to mammalian, especially human enzymes but have different specificities . Moreover, the invention provides libraries of specific engineered enzymes with corresponding specificities encoded genetically, a method for the generation of libraries of specific engineered enzymes with corresponding specificities encoded genetically, and the application of such libraries for technical, diagnostic, nutritional, personal care or research purposes.

A first aspect of the invention is directed to the application of engineered enzymes with specificities for therapeutic, research, diagnostic, nutritional, personal care or industrial purposes. The application comprises at least the following steps:

(a) identification of a target peptide substrate whose hydrolysis has a positive effect in connection with the intended purpose, such as curing a disease, diagnosing a disease, processing of ingredients for human or animal nutrition, or other technical processes;

(b) provision of an engineered enzyme, the enzyme being specific for the target peptide identified in step (a); and

(c) use of the enzyme as provided in step (b) for the intended purpose.

In a first variant of this aspect of the invention, the engineered enzyme is used as a therapeutic means to inactivate a disease-related target substrate. This application comprises at least the following steps:

(a) identification of a target substrate whose function is connected to a disease and whose inactivation has a positive effect in connection with the disease,

and determination of a target site within the target substrate characterized by the fact that modification at the target site leads to the inactivation of the target substrate;

(b) provision of an engineered enzyme, the enzyme being specific for the target site identified in step (a); and

(c) use of the enzyme for the inactivation of the target substrate inside or outside the human body.

Preferably, the scaffold is a protease and the modification is hydrolysis of a target site in a protein target. Preferably, the hydrolysis leads to the activation or inactivation of the peptide or protein target. Potential peptide or protein targets include soluble proteins, in particular cytokines, such as proteins of the TNF-superfamily, interleukines, interferons, chemokines and growth factors; hormones; toxins; enzymes, such as oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases; structural proteins, such as collagen; immunoglobulins; activity modulating proteins and DNA binding proteins; or membrane associated proteins, in particular single pass transmembrane proteins; multipass transmembrane proteins, such as G-protein coupled receptors, ion channels and transporters; lipid-anchored membrane proteins and GPI-anchored membrane proteins.

In a first embodiment of this variant the engineered enzyme is a protease and is capable of hydrolysing human tumor necrosis factor-alpha (hTNF-α). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, rheumatoid arthritis, inflammatory bowel diseases, psoriasis, Crohn's disease, Ulcerative colitis, diabetes type II, classical Hodgkin's Lymphoma (cHL), Grave's disease, Hashimoto's thyroiditis, Sjogren's Syndrome, systemic lupus erythematosus, multiple sclerosis, Systemic inflammatory response syndrome (SIRS) which leads to distant organ damage and multiple organ dysfunction syndrome (MODS), eosinophilia, neurodegenerative disease, stroke, closed head injury, encephalitis, CNS disorders, asthma, rheumatoid arthritis, sepsis, vasodilation, intravascular coagulation and multiple organ failure, as well as other diseases connected with hTNF-α. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hTNF-α (SEQ ID NO:96). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 31/32, 32/33, 44/45, 45/46, 87/88, 128/129, 130/131, 140/141 and/or 141/142 (most preferred between

positions 31/32, 32/33 and/or 45/46) in hTNF-α, or a peptide bond in proximity to these positions in hTNF-α, or peptide bonds in protein targets related to hTNF-α between positions having structural homology or sequence homology to these positions. In this embodiment it is most preferred that the protease has the a sequence shown in SEQ ID NO:74, SEQ ID NO:75 and is capable of hydrolysing hTNF-α at positions 31/32 and/or 32/33.

In a second embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Tumor necrosis factor ligand superfamily member 5 (hCD40-L). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, systemic lupus erythematosus and classical Hodgkin's Lymphoma (cHL), as well as other diseases connected with hCD40-L. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCD40-L (SEQ ID NO:143). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 133/134, 145/146, 165/166, 200/201, 201/202, 207/208 and/or 216/217 (most preferred between positions 133/134, 165/166, 201/202 and/or 216/217) in hCD40-L, or a peptide bond in proximity to these positions in hCD40-L, or peptide bonds in protein targets related to hCD40-L at positions having structural homology or sequence homology to these positions.

In a third embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Macrophage migration inhibitory factor (hMIF). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, inflammatory diseases, as well as other diseases connected with hMIF. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hMIF (SEQ ID NO:109). More preferably said engineered or said fusion protein is capable of hydrolysing the peptide bonds between positions 16/17, 44/45, 66/67, 73/74, 77/78, 88/89, 92/93 and/or 100/101 (most preferred between positions 16/17 and/or 92/93) in hMIF, or a peptide bond in proximity to these positions in hMIF, or peptide bonds in protein targets related to hMIF at positions having structural homology or sequence homology to these positions.

In a fourth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interleukin-1 beta precursor (hIL-1 beta). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, diabetes, brain inflammation in cancer,

arthritis, autoimmune and inflammatory diseases, as well as other diseases connected with hIL-1 beta. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-1 beta (SEQ ID NO:112). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 24/25, 35/36, 46/47, 54/55, 74/75, 75/76, 76/77, 77/78, 86/87, 88/89, 93/94, 94/95, 97/98 and/or 150/151 (most preferred between positions 35/36, 75/76, 76/77, 88/89, 93/94, 94/95 and/or 150/151) in hIL-1 beta, or a peptide bond in proximity to these positions in hIL-1 beta, or peptide bonds in protein targets related to hIL-1 beta at positions having structural homology or sequence homology to these positions.

In a fifth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interleukin 2 (hIL-2). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, T-cell leukemia and hairy cell leukemia, Crohn's disease, Ulcerative colitis, Grave's disease, Hashimoto's thyroiditis, Sjogren's syndrome, systemic lupus erythematosus, multiple sclerosis, asthma and chronic obstructive pulmonary and classical Hodgkin's Lymphoma (cHL), as well as other diseases connected with hIL-2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-2 (SEQ ID NO:99). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 20/21, 32/33, 38/39, 43/44, 45/46 48/49, 49/50, 54/55, 64/65, 76/77, 83/84, 84/85, 107/108, 109/110 and/or 120/121 (most preferred between positions 109/110) in hIL-2, or a peptide bond in proximity to these positions in hIL-2, or peptide bonds in protein targets related to hIL-2 at positions having structural homology or sequence homology to these positions.

In a sixth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interleukin 3 (hIL-3). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, classical Hodgkin's Lymphoma (cHL) and eosinophilia, as well as other diseases connected withh IL-3. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-3 (SEQ ID NO:148). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 21/22, 28/29, 36/37, 44/45, 46/47, 51/52, 63/64, 66/67, 79/80, 94/95, 101/102, 108/109 and/or 109/110 (most preferred between positions 21/22, 28/29, 46/47, 63/64, 66/67, 79/80 and/or 101/102) in hIL-3, or a peptide bond in

proximity to these positions in hIL-3, or peptide bonds in protein targets related to hIL-3 at positions having structural homology or sequence homology to these positions.

In a seventh embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interleukin 4 (hIL-4). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, classical Hodgkin's Lymphoma (cHL), Grave's disease, Hashimoto's thyroiditis, Sjogren's syndrome, Asthma, chronic obstructive pulmonary disease and allergic inflammatory reactions, as well as other diseases connected with hIL-4. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-4 (SEQ ID NO:118). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 4/5, 12/13, 31/32, 37/38, 61/62, 62/63, 64/65, 91/92, 102/103, 121/122 and/or 126/127 (most preferred between positions 4/5, 61/62, 62/63, 64/65 and/or 121/122) in hIL-4, or a peptide bond in proximity to these positions in hIL-4, or peptide bonds in protein targets related to hIL-4 at positions having structural homology or sequence homology to these positions.

In a eighth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interleukin-5 (hIL-5). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, classical Hodgkin's Lymphoma (cHL), asthma, chronic obstructive pulmonary disease, eosinophilia, allergic inflammatory diseases, as well as other diseases connected with hIL-5. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-5 (SEQ ID NO:133). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 12/13, 32/33, 67/68, 76/77, 77/78, 80/81, 83/84, 84/85, 85/86, 90/91, 91/92, 92/93 and/or 98/99 (most preferred between positions 90/91, 91/92, 92/93 and/or 98/99) in hIL-5, or a peptide bond in proximity to these positions in hIL-5, or peptide bonds in protein targets related to hIL-5 at positions having structural homology or sequence homology to these positions.

In a ninth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interleukin-6 (hIL-6). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, classical Hodgkin's Lymphoma (cHL), breast cancer, renal cell carcinoma, multiple myeloma, lymphoma, leukemia, Grave's disease, Hashimoto's

thyroiditis, Sjogren's syndrome, systemic lupus erythematosus, Systemic inflammatory response syndrome (SIRS) which leads to distant organ damage and multpile organ dysfunction syndrome (MODS), chronic obstructive pulmonary disease (COPD), Castleman's diseases, inflammatory bowel diseases, Crohn's disease, as well as other diseases connected with hIL-6. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-6 (SEQ ID NO:134). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 32/33, 35/36, 55/56, 71/72, 129/130, 130/131, 132/133, 135/136, 141/142, 161/162, 180/181 and/or 183/184 (most preferred between positions 135/136 and/or 141/142) in hIL-6, or a peptide bond in proximity to these positions in hIL-6, or peptide bonds in protein targets related to hIL-6 at positions having structural homology or sequence homology to these positions.

In a tenth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interleukin 8 (hIL-8). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, Crohn's disease, Ulcerative colitis, classical Hodgkin's Lymphoma (cHL), Systemic inflammatory response syndrome (SIRS) which leads to distant organ damage and multpile organ dysfunction syndrome (MODS), chronic obstructive pulmonary disease (COPD), endometriosis, psoriasis and atherosclerotic lesions, as well as other diseases connected with hIL-8. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-8 (SEQ ID NO:100). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 1/12, 15/16, 45/46, 47/48, 52/53, 54/55, 60/61, 64/65 and/or 67/68 (most preferred between positions 45/46) in hIL-8, or a peptide bond in proximity to these positions in hIL-8, or peptide bonds in protein targets related to hIL-8 at positions having structural homology or sequence homology to these positions.

In a eleventh embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interleukin-10 (hIL-10). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, classical Hodgkin's Lymphoma (cHL) and diseases related to the suppression of cytotoxic T-cells, as well as other diseases connected with hIL-10. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-10 (SEQ ID NO:135). More preferably said enzyme or said fusion protein is

capable of hydrolysing the peptide bonds between positions 24/25, 25/26, 27/28, 28/29, 40/41, 44/45, 49/50, 57/58, 59/60, 84/85, 86/87, 106/107, 107/108, 110/111, 130/131, 134/135, 137/138, 138/139 and/or 144/145 (most preferred between positions 24/25, 27/28, 44/45, 49/50, 86/87, 137/138 and/or 144/145) in hIL-10, or a peptide bond in proximity to these positions in hIL-10, or peptide bonds in protein targets related to hIL-10 at positions having structural homology or sequence homology to these positions.

In a twelfth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interleukin 12 beta chain (hIL-12ß). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, Crohn's disease and classical Hodgkin's Lymphoma (cHL), as well as other diseases connected with hIL-12ß. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-12ß (SEQ ID NO:97). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 14/15, 18/19, 29/30, 34/35, 87/88, 99/100, 102/103, 104/105, 161/162, 174/175, 222/223, 225/226, 228/229, 238/239, 268/269 and/or 293/294 (most preferred between positions 18/19, 34/35, 87/88 and/or 161/162) in hIL-12ß, or a peptide bond in proximity to these positions in hIL-12ß, or peptide bonds in protein targets related to hIL-12ß at positions having structural homology or sequence homology to these positions.

In a thirteenth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interleukin 13 (hIL-13). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of cancer, such as, but not limited to, classical Hodgkin's Lymphoma (cHL), eosinophilia, asthma, chronic obstructive pulmonary disease, fibrosis, psoriasis, atopic dermatitis and Ulcerative colitis, as well as other diseases connected with hIL-13. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-13 (SEQ ID NO:119). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 25/26, 62/63, 65/66, 86/87, 87/88, 98/99, 108/109 and/or 111/112 (most preferred between positions 87/88) in hIL-13, or a peptide bond in proximity to these positions in hIL-13, or peptide bonds in protein targets related to hIL-13 at positions having structural homology or sequence homology to these positions.

In a fourteenth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interleukin 18 (hIL-18). The enzymes or the fusion protein

can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, Crohn's disease, inflammation liver injuries, pulmonary tuberculosis, plural tuberculosis and rheumatoid arthritis, as well as other diseases connected with hIL-18. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-18 (SEQ ID NO:98). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 17/18, 32/33, 37/38, 39/40, 40/41, 53/54, 58/59, 79/80, 90/91, 93/94, 98/99, 110/111, 120/121, 123/124, 131/132, 132/133, 142/143, 147/148 and/or 157/158 (most preferred between positions 37/38, 132/133, 142/143 and/or 157/158) in hIL-18, or a peptide bond in proximity to these positions in hIL-18, or peptide bonds in protein targets related to hIL-18 at positions having structural homology or sequence homology to these positions.

In a fifteenth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interferon-gamma (hIFN-gamma). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, classical Hodgkin's Lymphoma (cHL), Crohn's disease and type I diabetes, as well as other diseases connected with hIFN-gamma. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIFN-gamma (SEQ ID NO:137). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 2/3, 6/7, 13/14, 21/22, 24/25, 34/35, 36/37, 37/38, 62/63, 68/69, 83/84, 86/87, 90/91, 102/103, 107/108 and/or 108/109 (most preferred between positions 13/14, 24/25, 37/38, 62/63, 68/69, 102/103 and/or 107/108) in hIFN-gamma, or a peptide bond in proximity to these positions in hIFN-gamma, or peptide bonds in protein targets related to hIFN-gamma at positions having structural homology or sequence homology to these positions.

In a sixteenth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human small inducible cytokine A2 (hCCL2). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, Crohn's disease and Ulcerative colitis, as well as other diseases connected with hCCL2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCCL2 (SEQ ID NO:102). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 3/4, 13/14, 18/19, 19/20, 24/25, 29/30, 38/39, 54/55, 56/57, 58/59, 62/63, 65/66 and/or 68/69 (most preferred between positions

19/20, 29/30, 38/39, 54/55 and/or 62/63) in hCCL2, or a peptide bond in proximity to these positions in hCCL2, or peptide bonds in protein targets related to hCCL2 at positions having structural homology or sequence homology to these positions.

In a seventeenth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Eotaxin (hCCL11). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, Crohn's disease and Ulcerative colitis, classical Hodgkin's Lymphoma (cHL), chronic pathophysiologic dysfunction, characterized by an influx mainly of Th2 cells, and eosinophilia, as well as other diseases connected with hCCL11. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCCL11 (SEQ ID NO:101). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 11/12, 16/17, 17/18, 22/23, 27/28, 33/34, 44/45, 47/48, 48/49, 52/53, 54/55, 56/57, 60/61, 66/67 and/or 73/74 (most preferred between positions 48/49 and/or 66/67) in hCCL11, or a peptide bond in proximity to these positions in hCCL11, or peptide bonds in protein targets related to hCCL11 at positions having structural homology or sequence homology to these positions.

In an eighteenth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Vascular endothelial growth factor (hVEGF). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, all solid tumors and metastatic solid tumors, inflammatory breast cancer, as well as other diseases connected with hVEGF. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hVEGF (SEQ ID NO:103). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 16/17, 19/20, 23/24, 34/35, 41/42, 56/57, 62/63, 63/64, 64/65, 65/66, 82/83, and/or 84/85 (most preferred between positions 23/24, 41/42, 63/64, 82/83 and/or 84/85) in hVEGF, or a peptide bond in proximity to these positions in hVEGF, or peptide bonds in protein targets related to hVEGF at positions having structural homology or sequence homology to these positions.

In an ninteenth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Transforming growth factor beta 1 (hTGF-ß1). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, a variety of cancers, including breast cancer, colorectal cancer and classical Hodgkin's Lymphoma (cHL), fibrosis,

suppression of cell-mediated immunity, glaucoma, diffuse systemic sclerosis as well as other diseases connected with hTGF-ß1. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hTGF-ß1 (SEQ ID NO:104). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 23/24, 25/26, 26/27, 27/28, 37/38, 55/56 and/or 94/95 (most preferred between positions 25/26, 55/56 and/or 94/95) in hTGF-ß1, or a peptide bond in proximity to these positions in hTGF-ß1, or peptide bonds in protein targets related to hTGF-ß1 at positions having structural homology or sequence homology to these positions.

In a twentieth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Somatotropin (human Growth hormone; hGH). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, acromegaly, diabetes and diabetic kidney disease including renal hypertrophy and glomerular enlargement and cardiovascular disorders, as well as other diseases connected with hGH. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hGH (SEQ ID NO:121). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 8/9, 16/17, 19/20, 26/27, 33/34, 38/39, 41/42, 70/71, 77/78, 94/95, 103/104, 112/113, 115/116, 116/117, 130/131, 147/148, 154/155 and/or 178/179 (most preferred between positions 112/113, 147/148 and/or 154/155) in hGH, or a peptide bond in proximity to these positions in hGH, or peptide bonds in protein targets related to hGH at positions having structural homology or sequence homology to these positions.

In a twenty-first embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Insulin-like growth factor II (hIGF-II). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, diabetes and diabetic kidney disease, as well as other diseases connected with hIGF-II. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIGF-II (SEQ ID NO:122). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 15/16, 23/24, 24/25, 34/35, 37/38, 38/39, 48/49 and/or 49/50 (most preferred between positions 23/24) in hIGF-II, or a peptide bond in proximity to these positions in hIGF-II, or peptide bonds in protein targets related to hIGF-II at positions having structural homology or sequence homology to these positions.

In a twenty-second embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Hepatocyte growth factor (hHGF). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, angiogenic disorders and hepatocellular carcinoma, as well as other diseases connected with hHGF. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hHGF (SEQ ID NO:120). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 54/55, 60/61, 62/63, 63/64, 68/69, 76/77, 112/113, 123/124, 134/135, 168/169, 198/199 and/or 202/203 (most preferred between positions 63/64, 68/69, 76/77, 168/169 and/or 202/203) in hHGF, or a peptide bond in proximity to these positions in hHGF, or peptide bonds in protein targets related to hHGF at positions having structural homology or sequence homology to these positions.

In a twenty-third embodiment of this variant the enzyme is a protease and is capable of hydrolysing human hInsulin (hInsulin). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, insulin overdosage, as well as other diseases connected with hInsulin. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hInsulin B chain (SEQ ID NO:105) and/or hInsulin A chain (SEQ ID NO:106). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 16/17 and/or 22/23 in hInsulin B and/or between position 14/15 in Insulin A, or a peptide bond in proximity to these positions in hInsulin A or B, or peptide bonds in protein targets related to hInsulin A or B at positions having structural homology or sequence homology to these positions.

In a twenty-fourth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human hGhrelin (hGhrelin). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, obesity, as well as other diseases connected with hGhrelin. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hGhrelin (SEQ ID NO:107). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 1/2, 2/3, 3/4 and/or 4/5 in hGhrelin, or a peptide bond in proximity to these positions in hGhrelin, or peptide bonds in protein targets related to hGhrelin at positions having structural homology or sequence homology to these positions.

In a twenty-fifth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human angiotensinogen (angiotensin). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, essential hypertension, as well as other diseases connected with angiotensin. Preferably, said enzyme or said fusion protein is capable of specifically inactivating angiotensin (SEQ ID NO:108). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 1/2, 3/4 and/or 7/8 (most preferred between positions 3/4) in angiotensin, or a peptide bond in proximity to these positions in angiotensin, or peptide bonds in protein targets related to angiotensin at positions having structural homology or sequence homology to these positions.

In a twenty-sixth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human leptin precursor (leptin). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, obesity, as well as other diseases connected with leptin. Preferably, said enzyme or said fusion protein is capable of specifically inactivating leptin (SEQ ID NO:127). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 8/9, 9/10, 15/16, 23/24, 40/41, 53/54, 71/72, 85/86, 94/95, 108/109 and/or 141/142 (most preferred between positions 9/10, 40/41, 71/72, 94/95 and/or 108/109) in leptin, or a peptide bond in proximity to these positions in leptin, or peptide bonds in protein targets related to leptin at positions having structural homology or sequence homology to these positions.

In a twenty-seventh embodiment of this variant the enzyme is a protease and is capable of hydrolysing Protective antigen (PA-83). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, anthrax infection, as well as other diseases connected with PA-83. Preferably, said enzyme or said fusion protein is capable of specifically inactivating PA-83 (SEQ ID NO:123). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 72/73, 73/74, 92/93, 93/94, 131/132, 149/150, 178/179, 213/214, 214/215, 387/388, 425/426, 426/427, 427/428, 453/454, 520/521, 608/609, 617/618, 671/672, 679/680, 680/681, 683/684 and/or 684/685 (most preferred between positions 72/73, 73/74, 93/94, 149/150, 387/388, 425/426, 427/428 and/or 683/684) in PA-83, or a peptide bond in proximity to these positions in PA-83, or peptide bonds in

protein targets related to PA-83 at positions having structural homology or sequence homology to these positions.

In a twenty-eighth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human plasminogen (plasminogen). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, thrombosis, as well as other diseases connected with plasminogen. Preferably, said enzyme or said fusion protein is capable of specifically inactivating plasminogen (SEQ ID NO:140). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bond between position 580/581 in plasminogen, or a peptide bond in proximity to this position in plasminogen, or peptide bonds in protein targets related to plasminogen at positions having structural homology or sequence homology to these positions.

In a twenty-ninth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Prothrombin (thrombin). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, bleeding, as well as other diseases connected with thrombin. Preferably, said enzyme or said fusion protein is capable of specifically inactivating thrombin (SEQ ID NO:149). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 198/199, 327/328, 363/364 (most preferred between positions 327/328 and/or 363/364) in thrombin, or a peptide bond in proximity to these positions in thrombin, or peptide bonds in protein targets related to thrombin at positions having structural homology or sequence homology to these positions

In a thirty embodiment of this variant the enzyme is a protease and is capable of hydrolysing human beta-secretase. The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, Alzheimer, as well as other diseases connected with human beta-secretase precursor. Preferably, said enzyme or said fusion protein is capable of specifically inactivating human beta-secretase precursor (SEQ ID NO:139). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 61/62, 64/65, 159/160, 238/239, 239/240, 246/247, 256/257, 330/331 and/or 365/366 (most preferred between positions 61/62, 246/247 and/or 365/366) in human beta-secretase precursor, or a peptide bond in proximity to these positions in human beta-secretase precursor, or peptide bonds in

protein targets related to human beta-secretase precursor at positions having structural homology or sequence homology to these positions.

In a thirty-first embodiment of this variant the enzyme is a protease and is capable of hydrolysing human matrix metalloproteinase-2 (hMMP-2). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, a variety of cancers including bladder cancer, breast tumor cancer, gastric cancer and lung cancer, as well as other diseases connected with hMMP-2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hMMP-2 (SEQ ID NO:131). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 62/63, 68/69, 75/76, 76/77, 79/80, 88/89, 110/111, 112/113, 115/116, 120/121, 164/165, 254/255, 267/268, 296/297, 324/325, 325/326, 382/383, 383/384, 470/471, 500/501, 550/551, 564/565, 595/596, 597/598, 608/609, 646/647, 649/650 and/or 650/651 (most preferred between positions 68/69, 115/116, 120/121, 164/165, 325/326, 383/384, 470/471, 500/501, 595/596, 608/609 and/or 650/651) in hMMP-2, or a peptide bond in proximity to these positions in hMMP-2, or peptide bonds in protein targets related to hMMP-2 at positions having structural homology or sequence homology to these positions.

In a thirty-second embodiment of this variant the enzyme is a protease and is capable of hydrolysing human matrix metalloproteinase-9 (hMMP-9). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, a variety of cancers including bladder cancer, breast tumor cancer, gastric cancer and lung cancer, as well as other diseases connected with hMMP-9. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hMMP-9 (SEQ ID NO:132). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 41/42, 42/43, 106/107, 113/114, 134/135, 160/161, 162/163, 163/164, 222/223, 226/227, 265/266, 266/267, 267/268, 284/285, 309/310, 321/322, 322/323, 324/325, 356/357, 380/381, 433/434 and/or 440/441 (most preferred between positions 160/161, 163/164, 226/227, 284/285, 321/322, 322/323 and/or 433/434) in hMMP-9, or a peptide bond in proximity to these positions in hMMP-9, or peptide bonds in protein targets related to hMMP-9 at positions having structural homology or sequence homology to these positions.

In a thirty-third embodiment of this variant the enzyme is a protease and is capable of hydrolysing HIV membrane glycoprotein (GP120). The enzymes or the fusion

protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, AIDS or HIV infection, as well as other diseases connected with GP120 or HIV infection. Preferably, said enzyme or said fusion protein is capable of specifically inactivating GP120 (SEQ ID NO:124). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 97/98, 99/100, 107/108, 113/114, 117/118, 227/228, 231/233, 279/280, 335/336, 337/338, 368/369, 412/413, 419/420, 429/430, 444/445, 457/458, 474/475, 476/477, 477/478, 485/486 and/or 490/491 (most preferred between positions 99/100, 368/369, 412/413, 419/420, 444/445 and/or 490/491) in GP120, or a peptide bond in proximity to these positions in GP120, or peptide bonds in protein targets related to GP120 at positions having structural homology or sequence homology to these positions.

In a thirty-fourth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Cytotoxic T-lymphocyte protein 4 (hCTLA-4). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, breast cancer, as well as other diseases connected with hCTLA-4. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCTLA-4 (SEQ ID NO:144). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 14/15, 28/29, 33/34, 38/39, 41/42, 62/63, 72/73, 85/86, 95/96, 100/101, 105/106, 119/120, 125/126 and/or 127/128 (most preferred between positions 14/15, 28/29, 38/39, 41/42, 62/63 and/or 85/86) in hCTLA-4, or a peptide bond in proximity to these positions in hCTLA-4, or peptide bonds in protein targets related to hCTLA-4 at positions having structural homology or sequence homology to these positions.

In a thirty-fifth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Integrin alpha-2 (hVLA-2). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, renal tumors, uveal melanomas and gastrointestinal tumors, as well as other diseases connected with hVLA-2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hVLA-2 (SEQ ID NO:147). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 160/161, 174/175, 201/202, 219/220, 231/232, 232/233, 233/234, 243/244, 259/260, 264/265, 268/269, 288/289, 292/293, 294/295, 298/299, 301/302, 310/311 and/or 317/318 (most preferred between positions

160/161, 174/175, 201/202, 219/220, 243/244, 264/265, 292/293 and/or 294/295) in hVLA-2, or a peptide bond in proximity to these positions in hVLA-2, or peptide bonds in protein targets related to hVLA-2 at positions having structural homology or sequence homology to these positions.

In a thirty-sixth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Vascular endothelial growth factor receptor 1 (hVEGFR 1). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, solid tumors and metastatic solid tumors, astrocytic brain tumors, pancreatic cancer, metastatic renal cancer, metastatic solid tumors, as well as other diseases connected with hVEGFR 1. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hVEGFR 1 (SEQ ID NO:114). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 189/190, 190/191, 224/225 and/or 331/332 (most preferred between positions 189/190 and/or 331/332) in hVEGFR 1, or a peptide bond in proximity to these positions in hVEGFR 1, or peptide bonds in protein targets related to hVEGFR 1 at positions having structural homology or sequence homology to these positions.

In a thirty-seventh embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Vascular endothelial growth factor receptor 2 (hVEGFR 2). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, solid tumors and metatstatic solid tumors, pancreatic cancer, metastatic renal cancer, metastatic CRC, as well as other diseases connected with hVEGFR 2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hVEGFR 2 (SEQ ID NO:115). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 214/215, and/or 323/324 (most preferred between position 214/215) in hVEGFR 2, or a peptide bond in proximity to these positions in hVEGFR 2, or peptide bonds in protein targets related to hVEGFR 2 at positions having structural homology or sequence homology to these positions.

In a thirty-eighth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Epidermal growth factor receptor (hEGFr). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of disesaes, such as, but not limited to, bladder cancer, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, oesophageal cancer, head

and neck cancer, gastric cancer, non-small-cell lung carcinoma and ovarian cancer, as well as other diseases connected with hEGFr. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hEGFr (SEQ ID NO:116). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 20/21, 29/30, 48/49, 74/75, 165/166, 202/203, 220/221, 246/247, 251/252, 269/270, 270/271, 304/305, 305/306, 357/358, 430/431, 443/444, 454/455, 455/456, 463/464, 465/466, 476/477, 507/508 and/or 509/510 in hEGFr, or a peptide bond in proximity to these positions in hEGFr, or peptide bonds in protein targets related to hEGFr at positions having structural homology or sequence homology to these positions.

In a thirty-ninth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Epithelial cell adhesion molecule (hEp-CAM). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, colorectal cancer, as well as other diseases connected with hEp-CAM. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hEp-CAM (SEQ ID NO:125). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 14/15, 19/20, 25/26, 30/31, 33/34, 55/56 an/or 70/71 (most preferred between positions 14/15, 30/31 and/or 70/71) in hEp-CAM, or a peptide bond in proximity to these positions in hEp-CAM, or peptide bonds in protein targets related to hEp-CAM at positions having structural homology or sequence homology to these positions.

In a forty embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Insulin-like growth factor I receptor (hIGF-1r). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, a variety of cancers including breast cancer, as well as other diseases connected with hIGF-1r. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIGF-1r (SEQ ID NO:126). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 59/60, 115/116, 146/147, 171/172, 191/192, 290/291, 306/307, 307/308, 335/336, 336/337, 455/456 and/or 470/471 (most preferred between positions 306/307, 307/308, 335/336 and/or 470/471) in hIGF-1r, or a peptide bond in proximity to these positions in hIGF-1r, or peptide bonds in protein targets related to hIGF-1r at positions having structural homology or sequence homology to these positions.

In a forty-first embodiment of this variant the enzyme is a protease and is capable of hydrolysing human T-cell surface antigen CD2 precursor (hCD2). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, psoriasis, as well as other diseases connected with hCD2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCD2 (SEQ ID NO:128). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 42/43, 43/44, 48/49, 49/50, 51/52, 54/55, 63/64, 69/70, 89/90 and/or 91/92 (most preferred between positions 43/44, 51/52 and/or 89/90) in hCD2, or a peptide bond in proximity to these positions in hCD2, or peptide bonds in protein targets related to hCD2 at positions having structural homology or sequence homology to these positions.

In a forty-second embodiment of this variant the enzyme is a protease and is capable of hydrolysing human T-cell surface glycoprotein CD4 (hCD4). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, psoriasis, transplant rejection, graft-versus-host colitis, autoimmune disorders and rheumatoid arthritis, as well as other diseases connected with hCD4. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCD4 (SEQ ID NO:129). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 166/167, 167/168, 206/207, 219/220, 224/225, 226/227, 251/252, 252/253, 322/323, 329/330 and/or 334/335 (most preferred between positions 206/207, 219/220, 251/252 and/or 252/253) in hCD4, or a peptide bond in proximity to these positions in hCD4, or peptide bonds in protein targets related to hCD4 at positions having structural homology or sequence homology to these positions.

In a forty-third embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Integrin alpha-L (hCD11a). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, psoriasis as well as other diseases connected with hCD11a. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCD11a (SEQ ID NO:130). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 145/146, 152/153, 156/157, 159/160, 160/161, 177/178, 178/179, 189/190, 190/191, 191/192, 193/194, 197/198, 200/201, 221/222, 229/230, 249/250, 253/254,

268/269, 290/291, 297/298, 304/305 and/or 305/306 (most preferred between positions 145/146, 159/160, 160/161, 189/190, 229/230, 249/250, 268/269, 297/298, 304/305 and/or 305/306) in hCD11a, or a peptide bond in proximity to these positions in hCD11a, or peptide bonds in protein targets related to hCD11a at positions having structural homology or sequence homology to these positions.

In a forty-fourth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Interferon-gamma receptor alpha chain (hIFN-gamma-R1). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, classical Hodgkin's Lymphoma (cHL) and type I diabetes, as well as other diseases connected with hIFN-gamma-R1. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIFN-gamma-R1 (SEQ ID NO:136). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 49/50, 52/53, 62/63, 106/107, 122/123, 174/175, 215/216 and/or 222/223 (most preferred between positions 49/50, 122/123, 174/175 and/or 215/216) in hIFN-gamma-R1, or a peptide bond in proximity to these positions in hIFN-gamma-R1, or peptide bonds in protein targets related to hIFN-gamma-R1 at positions having structural homology or sequence homology to these positions.

In a forty-fifth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Platelet membrane glycoprotein IIb/IIIa (hGPIIb/IIIa). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, unstable angina, carotid stenting, ischemic stroke, peripheral vascular diseases, angiogenesis-related diseases and disseminating tumors, as well as other diseases connected with hGPIIb/IIIa. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hGPIIb/IIIa (SEQ ID NO:141). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 67/68, 91/92, 129/130, 143/144, 144/145, 181/182, 208/209, 209/210, 216/217, 239/240, 261/262, 410/411, 532/533, 556/557, 557/558, 597/598, 650/651 and/or 689/690 (most preferred between positions 67/68, 261/262, 410/411, 650/651 and/or 689/690) in hGPIIb/IIIa, or a peptide bond in proximity to these positions in hGPIIb/IIIa, or peptide bonds in protein targets related to hGPIIb/IIIa at positions having structural homology or sequence homology to these positions.

In a forty-sixth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Intercellular adhesion molecule-1 (hICAM-1). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, Crohn's disease, as well as other diseases connected with hICAM-1. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hICAM-1 (SEQ ID NO:142). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 40/41, 88/89, 97/98, 102/103, 128/129, 131/132, 132/133, 149/150, 150/151, 160/161 and/or 166/167 (most preferred between positions 88/89, 102/103, 150/151, 160/161 and/or 166/167) in hICAM-1, or a peptide bond in proximity to these positions in hICAM-1, or peptide bonds in protein targets related to hICAM-1 at positions having structural homology or sequence homology to these positions.

In a forty-seventh embodiment of this variant the enzyme is a protease and is capable of hydrolysing human TGF-beta receptor type II (hTGF-beta RII). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, diffuse systemic sclerosis, as well as other diseases connected with hTGF-beta RII. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hTGF-beta RII (SEQ ID NO:145). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 32/33, 34/35, 35/36, 66/67, 67/68, 69/70, 82/83, 103/104, 104/105, 105/106, 118/119, 122/123 and/or 130/131 (most preferred between positions 32/33, 34/35, 66/67, 69/70, 104/105, 122/123 and/or 130/131) in hTGF-beta RII, or a peptide bond in proximity to these positions in hTGF-beta RII, or peptide bonds in protein targets related to hTGF-beta RII at positions having structural homology or sequence homology to these positions.

In a forty-eighth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Membrane cofactor protein (hMCP). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, renal tumors, uveal melanomas and gastrointestinal tumors, as well as other diseases connected with hMCP. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hMCP (SEQ ID NO:146). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 15/16, 17/18, 25/26, 31/32, 32/33, 35/36, 48/49, 67/68, 69/70, 110/111, 119/120 and/or 125/126 (most

preferred between positions 15/16, 32/33, 48/49, 119/120 and/or 125/126) 130/131) in hMCP, or a peptide bond in proximity to these positions in hMCP, or peptide bonds in protein targets related to hMCP at positions having structural homology or sequence homology to these positions.

In a forty-ninth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Protease activated receptor 1 (hPAR1). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, thrombosis, as well as other diseases connected with hPAR1. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hPAR1 (SEQ ID NO:110). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 46/47, 51/52 and/or 52/53 in PAR1, or a peptide bond in proximity to these positions in hPAR1, or peptide bonds in protein targets related to hPAR1 at positions having structural homology or sequence homology to these positions.

In a fifth embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Protease activated receptor 2 (hPAR2). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, Crohn's disease, Ulcerative colitis and Inflammatory bowel disease, asthma, inflammation associated pain and arthritis, as well as other diseases connected with hPAR2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hPAR2 (SEQ ID NO:111). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 41/42, 51/52 and/or 59/60 in hPAR2, or a peptide bond in proximity to these positions in hPAR2, or peptide bonds in protein targets related to hPAR2 at positions having structural homology or sequence homology to these positions.

In a fifty-first embodiment of this variant the enzyme is a protease and is capable of hydrolysing human Protease activated receptor 4 (hPAR4). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, thrombosis, as well as other diseases connected with hPAR4. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hPAR4 (SEQ ID NO:113). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 68/69, 74/75 and/or 78/79 in hPAR4, or a peptide bond in proximity to these

positions in hPAR4, or peptide bonds in protein targets related to hPAR4 at positions having structural homology or sequence homology to these positions.

In a fifty-second embodiment of this variant the enzyme is a protease and is capable of hydrolysing human 5-hydroxytryptamine 1A receptor (h5-HT-1A). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, irritable bowel syndrome, as well as other diseases connected with h5-HT-1A. Preferably, said enzyme or said fusion protein is capable of specifically inactivating h5-HT-1A (SEQ ID NO:117). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 101/102, 102/103, 181/182 and/or 370/371 in h5-HT-1A a peptide bond in proximity to these positions in h5-HT-1A, or peptide bonds in protein targets related to h5-HT-1A at positions having structural homology or sequence homology to these positions.

In a fifty-third embodiment of this variant the enzyme is a protease and is capable of hydrolysing human carcinoembryonic antigen (hCEA). The enzymes or the fusion protein can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, colon cancer, as well as other diseases connected with hCEA. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCEA (SEQ ID NO:138). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 17/18, 69/70, 71/72, 74/75, 77/78, 98/99, 116/117, 126/127 and/or 128/129 in hCEA, or a peptide bond in proximity to these positions in hCEA, or peptide bonds in protein targets related to hCEA at positions having structural homology or sequence homology to these positions.

In a fifty-fourth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human interleukin-1 receptor type 1 (hIL-1R). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, asthma, inflammation, rheumatic disorders, as well as other diseases connected with hIL-1R. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-1R (SEQ ID NO: 150). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 35/36, 42/43, 43/44, 44/45, 46/47, 56/57, 61/62, 72/73, 82/83, 98/99, 132/133, 137/138, 140/141, 145/146, 146/147, 148/149, 153/154, 171/172, 172/173, 190/191, 202/203, 203/204, 205/206, 242/243, 245/246, 251/252, 252/253, 253/254, 254/255, 261/262, 262/263, 265/266, 271/272,

272/273, 283/284, 285/286, 287/288, 290/291 and/or 298/299 (most preferred between positions 44/45, 46/47, 52/53, 61/62, 137/138, 148/149, 153/154, 171/172, 172/173, 203/204, 252/253, 253/254, 261/262, 271/272 and/or 290/291) in hIL-1R, or a peptide bond in proximity to these positions in hIL-1R, or peptide bonds in protein targets related to hIL-1R at positions having structural homology or sequence homology to these positions.

In a fifty-fifth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human interleukin-2 receptor beta chain (hIL-2Rb). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, acute myeloid leukemia, inflammation, psoriasis, as well as other diseases connected with hIL-2Rb. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-2Rb (SEQ ID NO: 151). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 38/39, 40/41, 41/42, 42/43, 43/44, 49/50, 71/72, 76/77, 81/82, 85/86, 86/87, 89/90, 91/92, 102/103, 105/106, 118/119, 134/135, 152/153, 153/154, 154/155, 161/162, 163/164, 165/166, 175/176, 194/195 and/or 197/198 (most preferred between positions 38/39, 43/44, 81/82, 118/119, 134/135, 153/154, 161/162, 165/166 and/or 194/195) in hIL-2Rb or a peptide bond in proximity to these positions in hIL-2Rb, or peptide bonds in protein targets related to hIL-2Rb at positions having structural homology or sequence homology to these positions.

In a fifty-sixth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human interleukin-4 receptor alpha chain (hIL-4Ra). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, asthma and allergy, as well as other diseases connected with hIL-4Ra. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hIL-4Ra (SEQ ID NO: 152). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 22/23, 32/33, 45/46, 52/53, 66/67, 67/68, 87/88, 112/113, 125/126, 127/128, 129/130, 141/142, 143/144, 148/149, 150/151, 154/155, 156/157, 160/161, 167/168, 173/174, 175/176, 177/178, 183/184 and/or 189/190 (most preferred between positions 52/53, 66/67, 112/113, 125/126, 143/144, 154/155 and/or 160/161) in hIL-4Ra or a peptide bond in proximity to these positions in hIL-4Ra, or peptide bonds in protein targets related to hIL-4Ra at positions having structural homology or sequence homology to these positions.

In a fifty-seventh embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human tumor necrosis factor receptor (hTNFR). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, asthma, Crohn´s disease, HIV infection, inflammation, psoriasis, rheumatoid arthritis, as well as other diseases connected with hTNFR. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hTNFR (SEQ ID NO: 153). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 40/41, 49/50, 51/52, 53/54, 54/55, 56/57, 68/69, 75/76, 77/78, 78/79, 79/80, 84/85, 91/92, 99/100, 100/101, 107/108, 109/110, 131/132, 132/133, 147/148, 149/150, 157/158, 158/159 and/or 161/162 (most preferred between positions 40/41, 49/50, 54/55, 78/79, 84/85, 99/100, 107/108, 109/110, 132/133, 149/150 and/or 157/158) in hTNFR or a peptide bond in proximity to these positions in hTNFR, or peptide bonds in protein targets related to hTNFR at positions having structural homology or sequence homology to these positions.

In a fifty-eighth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human C-X-C chemokine receptor type 5 (hCCR5). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, HIV infection, as well as other diseases connected with hCCR5. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCCR5 (SEQ ID NO: 154). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 8/9, 10/11, 12/13, 16/17, 19/20, 22/23, 23/24, 25/26, 27/28, 29/30, 34/35, 42/43, 50/51, 110/111, 115/116, 120/121, 123/124, 189/190, 201/202, 204/205, 207/208, 211/212, 215/216, 216/217, 219/220, 281/282, 285/286, 287/288, 290/291 and/or 294/295 in hCCR5 or a peptide bond in proximity to these positions in hCCR5, or peptide bonds in protein targets related to hCCR5 at positions having structural homology or sequence homology to these positions.

In a fifty-ninth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human C-X-C chemokine receptor type 3 (hCXCR3). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, multiple sclerosis, rheumatoid arthritis, as well as other diseases connected with hCXCR3. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCXCR3 (SEQ ID NO: 155). More preferably said enzyme or said fusion protein is capable of hydrolysing the

peptide bonds between positions 4/5, 7/8, 13/14, 21/22, 23/24, 27/28, 28/29, 29/30, 35/36, 46/47, 47/48, 52/53, 53/54, 112/113, 117/118, 119/120, 125/126, 195/196, 197/198, 205/206, 207/208, 212/213, 278/279, 282/283, 288/289, 292/293, 293/294, 295/296 and/or 297/298 in hCXCR3 or a peptide bond in proximity to these positions in hCXCR3, or peptide bonds in protein targets related to hCXCR3 at positions having structural homology or sequence homology to these positions.

In a sixty embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human epidermal growth factor (hEGF). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, carcinomas, solid cancers like breast, colon or stomach cancer, as well as other diseases connected with hEGF. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hEGF (SEQ ID NO: 156). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 11/12, 13/14, 17/18, 22/23, 27/28, 28/29, 40/41, 41/42, 44/45, 45/46, 46/47, 49/50 and/or 50/51 (most preferred between positions 11/12, 17/18, 44/45 an/or 49/50) in hEGF or a peptide bond in proximity to these positions in hEGF, or peptide bonds in protein targets related to hEGF at positions having structural homology or sequence homology to these positions.

In a sixty-first embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human fibroblast growth factor (hFGF-1). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancer, angiogenesis, as well as other diseases connected with hFGF-1. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hFGF-1 (SEQ ID NO: 157). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 28/29, 35/36, 36/37, 37/38, 39/40, 49/50, 60/61, 70/71, 74/75, 81/82, 94/95, 100/101, 101/102, 104/105, 105/106, 112/113, 113/114, 119/120, 122/123, 125/126 and/or 128/129 (most preferred between positions 28/29, 35/36, 70/71, 81/82, 100/101, 104/105, 113/114 adnd/or 122/123) in hFGF-1 or a peptide bond in proximity to these positions in hFGF-1, or peptide bonds in protein targets related to hFGF-1 at positions having structural homology or sequence homology to these positions.

In a sixty-second embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human fibroblast growth factor receptor 1 (hFGFR-1). The

enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancer, angiogenesis, as well as other diseases connected with hFGFR-1. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hFGFR-1 (SEQ ID NO: 158). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 15/16, 19/20, 22/23, 23/24, 24/25, 32/33, 49/50, 55/56, 56/57, 58/59, 60/61, 69/70, 70/71, 93/94, 95/96, 96/97, 110/111, 114/115, 134/135, 181/182, 182/183, 189/190; 194/195, 195/196 and/or 215/216 (most preferred between positions 19/20, 24/25, 49/50, 55/56, 58/59, 60/61, 95/96, 96/97, 110/111, 181/182, 189/190, 195/196 and/or 215/216) in hFGFR-1 or a peptide bond in proximity to these positions in hFGFR-1, or peptide bonds in protein targets related to hFGFR-1 at positions having structural homology or sequence homology to these positions.

In a sixty-third embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human fibroblast growth factor receptor 2 (hFGFR-2). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancers like astrocytomas, as well as other diseases connected with hFGFR-2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hFGFR-2 (SEQ ID NO: 159). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 9/10, 10/11, 14/15, 17/18, 18/19, 19/20, 32/33, 44/45, 50/51, 51/52, 53/54, 55/56, 62/63, 90/91, 91/92, 104/105, 105/106, 109/110, 127/128, 135/136, 149/150, 150/151, 175/176, 176/177, 177/178, 182/183, 189/190, 190/191 and/or 210/211 (most preferred between positions 14/15, 19/20, 53/54, 55/56, 91/92, 105/106, 149/150, 150/151, 175/176, 176/177, 182/183, 189/190 and/or 210/211) in hFGFR-2 or a peptide bond in proximity to these positions in hFGFR-2, or peptide bonds in protein targets related to hFGFR-2 at positions having structural homology or sequence homology to these positions.

In a sixty-fourth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human C-C chemokine receptor type 1 (hCCR1). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, multiple sclerosis, as well as other diseases connected with hCCR1. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCCR1 (SEQ ID NO: 160). More

preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 2/3, 8/9, 9/10, 10/11, 11/12, 15/16, 17/18, 18/19, 26/27, 29/30, 30/31, 32/33, 92/93, 93/94, 94/95, 96/97, 97/98, 98/99, 99/100, 101/102, 103/104, 107/108, 173/174, 176/177, 177/178, 178/179, 187/188, 190/191, 193/194, 194/195, 195/196, 196/197, 266/267, 272/273, 274/275, 277/278 and/or 280/281 in hCCR1 or a peptide bond in proximity to these positions in hCCR1, or peptide bonds in protein targets related to hCCR1 at positions having structural homology or sequence homology to these positions.

In a sixty-fifth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human C-C chemokine receptor type 2 (hCCR2). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, multiple sclerosis, rheumatoid arthritis, as well as other diseases connected with hCCR2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCCR2 (SEQ ID NO: 161). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 6/7, 8/9, 9/10, 11/12, 15/16, 18/19, 19/20, 23/24, 25/26, 27/28, 34/35, 36/37, 38/39, 105/106, 106/107, 108/109, 114/115, 180/181, 183/184, 184/185, 185/186, 188/189, 193/194, 194/195, 196/197, 198/199, 201/202, 206/207, 270/271, 271/272, 272/273, 278/279 and/or 284/285 in hCCR2 or a peptide bond in proximity to these positions in hCCR2, or peptide bonds in protein targets related to hCCR2 at positions having structural homology or sequence homology to these positions.

In a sixty-sixth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human tyrosine protein kinase (hSrc). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancer, osteoporosis, as well as other diseases connected with hSrc. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hSrc (SEQ ID NO: 162). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 15/16, 22/23, 25/26, 59/60, 65/66, 87/88, 94/95, 99/100, 102/103, 123/124, 124/125, 126/127, 135/136, 147/148, 150/151, 153/154, 158/159, 176/177, 178/179, 182/183, 200/201, 216/217, 223/224, 234/235, 249/250, 261/262, 266/267, 271/272, 275/276, 277/278, 297/298, 327/328, 331/332, 333/334, 337/338, 354/355, 356/357, 378/379, 387/388, 397/398, 391/392, 395/396, 398/399, 407/408, 411/412, 418/419, 419/420, 420/421, 422/423, 423/424

and/or 436/437 (most preferred between positions 59/60, 123/124, 126/127, 135/136, 176/177, 182/183, 200/201, 275/276, 277/278, 331/332, 354/355, 387/388, 391/392, 395/396, 418/419 and/or 423/424) in hSrc or a peptide bond in proximity to these positions in hSrc, or peptide bonds in protein targets related to hSrc at positions having structural homology or sequence homology to these positions.

In a sixty-seventh embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human RAC-beta serine/threonine protein kinase (hAkt-2). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancer, as well as other diseases connected with hAkt-2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hAkt-2 (SEQ ID NO: 163). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 9/10, 15/16, 25/26, 26/27, 27/28, 39/40, 63/64, 71/72, 72/73, 78/79, 79/80, 98/99, 99/100, 100/101, 104/105, 105/106, 106/107, 120/121, 124/125, 125/126, 141/142, 170/171, 178/179, 179/180, 181/182, 182/183, 184/185, 206/207, 209/210, 211/212, 212/213, 220/221, 221/222, 223/224, 226/227, 242/243, 243/244, 245/246, 256/257, 260/261, 262/263, 275/276, 276/277, 282/283, and /or 292/293 (most preferred between positions 27/28, 39/40, 41/42, 72/73, 78/79, 100/101, 105/106, 106/107, 125/126, 179/180, 184/185, 209/210, 223/224, 245/246, 262/263 and/or 282/283) in hAkt-2 or a peptide bond in proximity to these positions in hAkt-2, or peptide bonds in protein targets related to hAkt-2 at positions having structural homology or sequence homology to these positions.

In a sixty-eighth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human substance P (substance P). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancers like small cell lung cancer, colorectal cancer, astrocytic/glial brain tumors, as well as other diseases connected with substance P. Preferably, said enzyme or said fusion protein is capable of specifically inactivating substance P (SEQ ID NO: 164). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 1/2, 3/4, 7/8 and/or 8/9 in substance P or a peptide bond in proximity to these positions in substance P, or peptide bonds in protein targets related to substance P at positions having structural homology or sequence homology to these positions.

In a sixty-ninth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human Bradykinin (Bradykinin). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, vascular and neuro-glial pathology in diabetic retinopathy, cerebral ischemia and trauma, hyperalgesia, inflammatory diseases or conditions, asthma and cancer, pain, pathological vascular leakage or vasodilation, pathological contraction of various smooth muscles, pathological cell proliferation, as well as other diseases connected with Bradykinin. Preferably, said enzyme or said fusion protein is capable of specifically inactivating Bradykinin (SEQ ID NO: 165). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 1/2, 5/6 and/or 8/9 in Bradykinin or a peptide bond in proximity to these positions in Bradykinin, or peptide bonds in protein targets related to Bradykinin at positions having structural homology or sequence homology to these positions.

In a seventy embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human Coagulation factor IX (Factor IX). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, hämophilia B, as well as other diseases connected with Factor IX. Preferably, said enzyme or said fusion protein is capable of specifically inactivating Factor IX (SEQ ID NO: 166). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 21/22, 23/24, 36/37, 38/39, 59/60, 63/64, 74/75, 75/76, 87/88, 111/112, 112/113, 119/120, 127/128, 128/129, 129/130, 130/131, 136/137, 137/138, 149/150,151/152, 153/154, 162/163, 167/168, 173/174, 176/177, 190/191, 209/210, 222/223, 223/224 and/or 227/228 (most preferred between positions 63/64, 127/128, 136/137, 149/150, 151/152, 173/174, 176/177 and/or 227/228) in Factor IX or a peptide bond in proximity to these positions in Factor IX, or peptide bonds in protein targets related to Factor IX at positions having structural homology or sequence homology to these positions.

In a seventy-first embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human glycogen synthase kinase-3-beta (hGSK-3). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, diabetes, as well as other diseases connected with hGSK-3. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hGSK-3 (SEQ ID NO: 167). More preferably said

enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 16/17, 19/20, 24/25, 26/27, 43/44, 52/53, 57/58, 60/61, 62/63, 68/69, 69/70, 87/88, 88/89, 89/90, 90/91, 91/92, 107/108, 110/111, 112/113, 114/115, 116/117, 156/157, 158/159, 175/176, 177/178, 182/183, 186/187, 187/188, 189/190, 226/227, 230/231, 234/235, 244/245, 245/246, 248/249, 249/250, 254/255, 256/257, 263/264, 269/270, 272/273, 274/275, 307/308, 308/309, 311/312, 315/316 and/or 321/322 (most preferred between positions 57/58, 87/88, 88/89, 89/90, 90/91, 114/115, 116/117, 158/159, 175/176, 182/183, 230/231, 244/245, 248/249, 254/255, 256/257, 274/275 and/or 321/322) in hGSK-3 or a peptide bond in proximity to these positions in hGSK-3, or peptide bonds in protein targets related to hGSK-3 at positions having structural homology or sequence homology to these positions.

In a seventy-second embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human cyclin-dependent protein kinase-2 (hcdk-2). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancer, as well as other diseases connected with hcdk-2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hcdk-2 (SEQ ID NO: 168). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 8/9, 9/10, 12/13, 15/16, 19/20, 22/23, 24/25, 34/35, 50/51, 57/58, 68/69, 73/74, 75/76, 88/89, 89/90, 92/93, 122/123, 138/139, 162/163, 178/179, 179/180, 180/181, 199/200, 200/201, 206/207, 208/209, 210/211, 217/218, 223/224, 224/225, 237/238, 242/243, 245/246, 247/248, 250/251, 273/274 and/or 291/292 (most preferred between positions 12/13, 50/51, 57/58, 73/74, 138/139, 180/181, 200/201, 206/207, 223/224, 242/243, 247/248 and/or 273/274) in hcdk-2 or a peptide bond in proximity to these positions in hcdk-2, or peptide bonds in protein targets related to hcdk-2 at positions having structural homology or sequence homology to these positions.

In a seventy-third embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human caspase-2 (caspase-2). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, pathological ischemic cell death, pathological reperfusion cell death, pathological retinal neuronal cell death, pathological

apoptosis initiated by beta-amyloid toxicity or by trophic factor deprivation, diseases with mitochondrial permeabilization components, toxin cell death induced by cytolethal distending toxin (CDT), acute ischemic injury, infertility, wounds, as well as other diseases connected with caspase-2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating caspase-2 (SEQ ID NO: 169). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 13/14, 14/15, 24/25, 28/29, 44/45, 45/46, 46/47, 48/49, 54/55, 65/66, 70/71, 81/82, 84/85, 96/97, 118/119, 120/121, 126/127, 154/155, 155/156, 186/187, 188/189, 212/213, 213/214, 227/228, 228/229, 247/248, 249/250, 251/252, 259/260 and/or 272/273 (most preferred between positions 48/49, 81/82, 154/155, 186/187, 213/214, 228/229 and/or 251/252) in caspase-2 or a peptide bond in proximity to these positions in caspase-2, or peptide bonds in protein targets related to caspase-2 at positions having structural homology or sequence homology to these positions.

In a seventy-fourth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human caspase-3 (caspase-3). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic acell death, reperfusion cell death, acute ischemic injury, infertility, wounds, neural degeneration in amyotrophic lateral sclerosis, Huntington, Infection with vesicular stomatitis virus (VSV), as well as other diseases connected with caspase-3. Preferably, said enzyme or said fusion protein is capable of specifically inactivating caspase-3 (SEQ ID NO: 170). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 12/13, 25/26, 29/30, 40/41, 47/48, 48/49, 51/52, 54/55, 56/57, 58/59, 66/67, 67/68, 73/74, 74/75, 77/78, 78/79, 79/80, 82/83, 83/84, 110/111, 139/140, 151/152, 152/153, 153/154, 158/159, 196/197, 198/199, 200/201, 201/202, 218/219, 220/221, 225/226 and/or 248/249 (most preferred between positions 29/30, 40/41, 51/52, 56/57, 67/68, 73/74, 79/80, 83/84, 153/154, 218/219 and/or 225/226) in caspase-3 or a peptide bond in proximity to these positions in caspase-3, or peptide bonds in protein targets related to caspase-3 at positions having structural homology or sequence homology to these positions.

In a seventy-fifth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human caspase-7 (caspase-7). The enzymes or the fusion

proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic acell death, reperfusion cell death, acute ischemic injury, infertility, wounds, toxin cell death induced by cytolethal distending toxin (CDT), acute lymphoblast leukemia, as well as other diseases connected with caspase-7. Preferably, said enzyme or said fusion protein is capable of specifically inactivating caspase-7 (SEQ ID NO: 171). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 35/36, 42/43, 43/44, 56/57, 57/58, 68/69, 72/73, 76/77, 79/80, 84/85, 88/89, 101/102, 102/103, 105/106, 106/107, 107/108, 149/150, 188/189, 189/190, 227/228, 228/229, 231/232, 232/233, 251/252, 255/256, 256/257 and/or 277/278 (most preferred between positions 57/58, 79/80, 84/85, 102/103, 107/108, 228/229, 231/232, 232/233 and/or 255/256) in caspase-7 or a peptide bond in proximity to these positions in caspase-7, or peptide bonds in protein targets related to caspase-7 at positions having structural homology or sequence homology to these positions.

In a seventy-sixth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human caspase-9 (caspase-9). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic acell death, reperfusion cell death, acute ischemic injury, infertility, wounds, neurological diseases like stroke, neurodegenerative diseases, brain injury caused by hypoxia, Parkinson's, amyotrophic lateral sclerosis (ALS), as well as other diseases connected with caspase-9. Preferably, said enzyme or said fusion protein is capable of specifically inactivating caspase-9 (SEQ ID NO: 172). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 4/5, 19/20, 34/35, 35/36, 39/40, 49/50, 52/53, 53/54, 54/55, 63/64, 67/68, 71/72, 72/73, 79/80, 84/85, 112/113, 123/124, 151/152, 158/159, 215/216, 216/217, 217/218, 219/220, 223/224, 226/227, 229/230, 230/231, 233/234, 235/236 and/or 258/259 (most preferred between positions 19/20, 35/36, 34/35, 52/53, 53/54, 71/72, 79/80, 219/220 and/or 230/231) in caspase-9 or a peptide bond in proximity to these positions in caspase-9, or peptide bonds in protein targets

related to caspase-9 at positions having structural homology or sequence homology to these positions.

In a seventy-seventh embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human apoptotic protease activating factor 1 (hApaf-1). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic acell death, reperfusion cell death, acute ischemic injury, infertility, wounds, as well as other diseases connected with hApaf-1. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hApaf-1 (SEQ ID NO: 173). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 6/7, 13/14, 14/15, 17/18, 18/19, 19/20, 24/25, 27/28, 32/33, 39/40, 40/41, 41/42, 44/45, 46/47, 62/63, 63/64, 64/65, 66/67, 80/81, 81/82 and/or 82/83 (most preferred between positions 13/14, 14/15, 18/19, 41/42, 62/63 and/or 64/65) in hApaf-1or a peptide bond in proximity to these positions in hApaf-1, or peptide bonds in protein targets related to hApaf-1 at positions having structural homology or sequence homology to these positions.

In a seventy-eighth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human BH3 interacting domain death agonist (hBID). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic acell death, reperfusion cell death, acute ischemic injury, infertility, wounds, as well as other diseases connected with hBID. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hBID (SEQ ID NO: 174). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 6/7, 15/16, 32/33, 36/37, 37/38, 38/39, 53/54, 54/55, 56/57, 57/58, 58/59, 62/63, 65/66, 73/74, 77/78, 79/80, 101/102, 116/117, 120/121, 122/123, 123/124, 124/125, 134/135, 140/141, 142/143, 143/144, 145/146 and/or 170/171 (most preferred between positions 36/37, 53/54, 57/58, 62/63, 65/66, 73/74 and/or 79/80) in hBID or a peptide bond in proximity to these positions in hBID, or peptide bonds in protein targets related to hBID at positions having structural homology or sequence homology to these positions.

In a seventy-ninth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human poly (ADP-ribose) polymerase-1 (hPARP). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic acell death, reperfusion cell death, acute ischemic injury, infertility, wounds, as well as other diseases connected with hPARP. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hPARP (SEQ ID NO: 175). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 13/14, 19/20, 22/23, 23/24, 27/28, 29/30, 34/35, 39/40, 42/43, 43/44, 65/66, 70/71, 74/75, 82/83, 86/87, 87/88, 114/115, 118/119, 122/123, 126/127, 133/134, 134/135, 141/142, 144/145, 145/146, 146/147, 148/149, 149/150, 158/159, 179/180, 181/182, 188/189, 196/197, 222/223, 270/271, 272/273, 282/283, 304/305, 307/308 and/or 320/321 (most preferred between positions 22/23, 43/44, 118/119, 122/123, 145/146, 146/147, 148/149, 179/180 and/or 272/273) in hPARP or a peptide bond in proximity to these positions in hPARP, or peptide bonds in protein targets related to hPARP at positions having structural homology or sequence homology to these positions.

In an eighty embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human Tumor protein p53 (hp53). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, any degenerative disorders (neurodegenerative diseases), ischemic and reperfusion cell death, acute ischemic injury, infertility, wounds, as well as other diseases connected with hp53. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hp53 (SEQ ID NO: 176). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 8/9, 10/11, 14/15, 17/18, 27/28, 33/34, 53/54, 55/56, 63/64, 78/79, 81/82, 87/88, 88/89, 93/94, 105/106, 109/110, 112/113, 114/115, 115/116, 116/117, 131/132, 135/136, 155/156, 156/157, 166/167, 180/181, 187/188, 189/190, 190/191, 192/193, 193/194 and/or 194/195 (most preferred between positions 14/15, 27/28, 53/54, 88/89, 114/115, 116/117, 131/132, 155/156, 190/191 and/or 194/195) in hp53 or a peptide bond in proximity to these positions in hp53, or peptide bonds in protein

targets related to hp53 at positions having structural homology or sequence homology to these positions.

In an eighty-first embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human P-selectin (hP-selectin). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, inflammation, as well as other diseases connected with hP-selectin. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hP-selectin (SEQ ID NO: 177). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 8/9, 16/17, 17/18, 18/19, 22/23, 23/24, 36/37, 37/38, 40/41, 44/45, 45/46, 54/55, 55/56, 66/67, 67/68, 72/73, 74/75, 78/79, 80/81, 84/85, 85/86, 88/89, 92/93, 94/95, 96/97, 106/107, 107/108, 111/112, 112/113, 124/125, 129/130, 140/141, 152/153 and/or 154/155 (most preferred between positions 17/18, 22/23, 44/45, 55/56, 72/73, 78/79, 84/85, 85/86, 107/108, 112/113, 152/153 and/or 154/155) in hP-selectin or a peptide bond in proximity to these positions in hP-selectin, or peptide bonds in protein targets related to hP-selectin at positions having structural homology or sequence homology to these positions.

In an eighty-second embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human Oncostatin M (hOSM). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancer like prostate cancer, as well as other diseases connected with hOSM. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hOSM (SEQ ID NO: 178). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 11/12, 19/20, 22/23, 26/27, 32/33, 36/37, 41/42, 44/45, 46/47, 47/48, 50/51, 52/53, 59/60, 60/61, 67/68, 68/69, 84/85, 97/98, 99/100, 100/101, 106/107, 107/108, 109/110, 122/123, 126/127, 133/134, 158/159, 162/163, 163/164 and/or 175/176 (most preferred between positions 19/20, 44/45, 47/48, 60/61, 67/68, 97/98, 100/101, 109/110, 126/127, 133/134, 162/163 and/or 175/176) in hOSM or a peptide bond in proximity to these positions in hOSM, or peptide bonds in protein targets related to hOSM at positions having structural homology or sequence homology to these positions.

In an eighty-third embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human cathepsin B (cathepsin B). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases,

such as, but not limited to, inflammatory bowel disease, Crohn´s disease, colitis ulcerosa, as well as other diseases connected with cathepsin B. Preferably, said enzyme or said fusion protein is capable of specifically inactivating cathepsin B (SEQ ID NO: 179). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 8/9, 9/10, 18/19, 53/54, 69/70, 75/76, 78/79, 85/86, 86/87, 94/95, 95/96, 124/125, 127/128, 130/131, 141/142, 146/147, 148/149, 151/152, 158/159, 159/160, 165/166, 166/167, 184/185, 194/195, 224/225, 227/228, 238/239, 245/246 and/or 252/253 (most preferred between positions 75/76, 85/86, 95/96, 124/125, 130/131, 141/142, 148/149, 158/159 and/or 194/195) in cathepsin B or a peptide bond in proximity to these positions in cathepsin B, or peptide bonds in protein targets related to cathepsin B at positions having structural homology or sequence homology to these positions.

In an eighty-fourth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human cathepsin D (cathepsin D). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, inflammatory bowel disease, Crohn´s disease, colitis ulcerosa, as well as other diseases connected with cathepsin D. Preferably, said enzyme or said fusion protein is capable of specifically inactivating cathepsin D (SEQ ID NO: 180). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 10/11, 18/19, 47/48, 54/55, 58/59, 62/63, 63/64, 67/68, 69/70, 75/76, 86/87, 111/112, 112/113, 141/142, 158/159, 161/162, 172/173, 174/175, 189/190, 191/192, 192/193, 197/198, 202/203, 203/204, 214/215, 223/224, 224/225, 227/228, 242/243, 243/244, 245/246, 246/247, 249/250, 266/267, 281/282, 283/284, 284/285, 288/289, 289/290, 293/294, 299/300, 310/311 and/or 336/337 (most preferred between positions 54/55, 62/63, 63/64, 112/113, 158/159, 174/175, 189/190, 197/198, 224/225, 242/243, 245/246, 266/267, 281/282, 288/289 and/or 299/300) in cathepsin D or a peptide bond in proximity to these positions in cathepsin D, or peptide bonds in protein targets related to cathepsin D at positions having structural homology or sequence homology to these positions.

In an eighty-fifth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human cathepsin L (cathepsin L). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, inflammatory bowel disease, Crohn´s disease, colitis ulcerosa, as well as other diseases connected with cathepsin L. Preferably, said

enzyme or said fusion protein is capable of specifically inactivating cathepsin L (SEQ ID NO: 181). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 9/10, 10/11, 40/41, 41/42, 44/45, 72/73, 76/77, 79/80, 86/87, 87/88, 95/96, 96/97, 99/100, 103/104, 104/105, 114/115, 117/118, 120/121, 124/125, 141/142, 148/149, 155/156, 159/160, 160/161, 182/183, 189/190, 193/194, 198/199, 191/192, 192/193, 205/206 and/or 206/207 (most preferred between positions 40/41, 87/88, 95/96, 103/104, 120/121, 141/142, 155/156, 159/160, 192/193 and/or 206/207) in cathepsin L or a peptide bond in proximity to these positions in cathepsin L, or peptide bonds in protein targets related to cathepsin L at positions having structural homology or sequence homology to these positions.

In an eighty-sixth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human Galectin-3 (hGalectin-3). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, inflammatory bowel disease, Crohn´s disease, colitis ulcerosa, as well as other diseases connected with hGalectin-3. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hGalectin-3 (SEQ ID NO: 182). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 5/6, 16/17, 26/27, 31/32, 35/36, 52/53, 55/56, 56/57, 65/66, 68/69, 70/71, 71/72, 72/73, 73/74, 80/81, 83/84, 92/93, 97/98, 102/103, 111/112, 113/114, 114/115,126/127, 128/129 and/or 134/135 (most preferred between positions 55/56, 65/66, 70/71, 102/103, 113/114 and/or 128/129) in hGalectin-3or a peptide bond in proximity to these positions in hGalectin-3, or peptide bonds in protein targets related to hGalectin-3 at positions having structural homology or sequence homology to these positions.

In an eighty-seventh embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human receptor tyrosine-protein kinase erbB-2 (hHER2). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancer, as well as other diseases connected with hHER2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hHER2 (SEQ ID NO: 183). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 18/19, 70/71, 87/88, 88/89, 99/100, 116/117, 135/136, 143/144, 153/154, 163/164, 168/169, 188/189, 206/207, 216/217, 226/227, 252/253, 255/256, 258/259, 264/265, 266/267, 279/280, 311/312, 314/315, 318/319,

326/327, 330/331, 332/333, 357/358, 360/361, 373/374, 395/396, 477/478, 479/480, 480/481, 481/482, 485/486, 495/496, 514/515, 520/521, 521/522, 523/524, 530/531, 532/533, 536/537, 558/559, 560/561, 568/569, 577/578, 592/593, 597/598 and/or 598/599 (most preferred between positions 88/89, 135/136, 143/144, 226/227, 252/253, 255/256, 258/259, 314/315, 318/319, 360/361, 480/481, 485/486, 495/496, 520/521, 523/524, 560/561, 568/569, 577/578 and/or 592/593) in hHER2 or a peptide bond in proximity to these positions in hHER2, or peptide bonds in protein targets related to hHER2 at positions having structural homology or sequence homology to these positions.

In an eighty-eighth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human matrix metalloproteinase-7 (hMMP-7). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancer, as well as other diseases connected with hMMP-7. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hMMP-7 (SEQ ID NO: 184). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 13/14, 22/23, 24/25, 25/26, 33/34, 37/38, 44/45, 45/46, 51/52, 52/53, 55/56, 66/67, 73/74, 76/77, 100/101, 101/102, 102/103, 103/104, 106/107, 133/134, 146/147, 151/152, 155/156, 162/163 and/or 166/167 (most preferred between positions 24/25, 33/34, 51/52, 55/56, 73/74, 76/77, 101/102, 133/134 and/or 146/147) in hMMP-7 or a peptide bond in proximity to these positions in hMMP-7, or peptide bonds in protein targets related to hMMP-7 at positions having structural homology or sequence homology to these positions.

In an eighty-ninth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human matrix metalloproteinase-14 (hMMP-14). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancer, as well as other diseases connected with hMMP-14. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hMMP-14 (SEQ ID NO: 185). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 12/13, 20/21, 26/27, 27/28, 34/35, 35/36, 38/39, 47/48, 49/50, 57/58, 50/51, 53/54, 55/56, 58/59, 62/63, 72/73, 82/83, 84/85, 113/114, 115/116, 116/117, 141/142, 152/153, 154/155, 156/157, 165/166 and/or 166/167 (most preferred between positions 27/28, 38/39, 55/56, 57/58, 58/59, 82/83, 113/114, 116/117, 141/142 and/or 152/153) in hMMP-14 or a peptide bond in proximity to

these positions in hMMP-14, or peptide bonds in protein targets related to hMMP-14 at positions having structural homology or sequence homology to these positions.

In a ninety embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human Vascular endothelial growth factor receptor 2 (hVEGFR-2). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, cancer, as well as other diseases connected with hVEGFR-2. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hVEGFR-2 (SEQ ID NO: 186). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 13/14, 16/17, 19/20, 23/24, 33/34, 39/40, 53/54, 61/62, 88/89, 110/111, 112/113, 113/114, 115/116, 119/120, 120/121, 179/180, 184/185, 198/199, 203/204, 204/205, 208/209, 220/221, 245/246, 256/257, 260/261, 261/262, 291/292, 293/294, 294/295, 295/296, 298/299, 299/300, 301/302, 302/303, 307/308, 310/311, 311/312, 317/318, 322/323, 327/328, 336/337 and/or 339/340 (most preferred between positions 39/40, 53/54, 61/62, 88/89, 119/120, 120/121, 204/205, 260/261, 293/294, 298/299, 299/300, 302/303 and/or 336/337) in hVEGFR-2 or a peptide bond in proximity to these positions in hVEGFR-2, or peptide bonds in protein targets related to hVEGFR-2 at positions having structural homology or sequence homology to these positions.

In a ninety-first embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human Mitogen-activated protein kinase p38-alpha (hp38-kinase). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, intimal hyperplasia, vascular remodeling upon blood vessel injury, as well as other diseases connected with hp38-kinase. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hp38-kinase (SEQ ID NO: 187). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 8/9, 11/12, 14/15, 21/22, 48/49, 53/54, 56/57, 66/67, 93/94, 96/97, 97/98, 117/118, 120/121, 123/124, 124/125, 159/160, 160/161, 162/163, 172/173, 175/176, 176/177, 177/178, 181/182, 199/200, 219/220, 229/230, 232/233, 236/237, 244/245, 247/248, 248/249, 252/253, 255/256, 257/258, 285/286, 286/287, 293/294, 294/295, 310/311, 312/313, 314/315, 315/316, 316/317, 320/321, 323/324, 329/330, 330/331, 334/335, 335/336, 341/342, 342/343 and/or 343/344 (most preferred between positions 48/49, 56/57, 93/94, 96/97, 123/124, 160/161, 175/176, 236/237, 247/248, 255/256, 257/258, 294/295, 314/315, 315/316,

329/330, 330/331, 334/335 and/or 342/343) in hp38-kinase or a peptide bond in proximity to these positions in hp38-kinase, or peptide bonds in protein targets related to hp38-kinase at positions having structural homology or sequence homology to these positions.

In a ninety-second embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human Stress-activated protein kinase JNK3 (hJNK3-kinase). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, intimal hyperplasia, vascular remodeling upon blood vessel injury, as well as other diseases connected with hJNK3-kinase. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hJNK3-kinase (SEQ ID NO: 188). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 11/12, 19/20, 38/39, 43/44, 44/45, 53/54, 65/66, 72/73, 90/91, 93/94, 94/95, 106/107, 116/117, 118/119, 120/121, 124/125, 134/135, 179/180, 196/197, 197/198, 198/199, 214/215, 216/217, 222/223, 223/224, 233/234, 244/245, 245/246, 251/252, 253/254, 255/256, 259/260, 267/268, 271/272, 277/278, 279/280, 282/283, 302/303, 307/308, 308/309, 318/319, 319/320, 325/326, 338/339, 339/340, 344/345 and/or 345/346 (most preferred between positions 38/39, 90/91, 93/94, 116/117, 179/180, 216/217, 222/223, 255/256, 259/260, 267/268, 271/272, 277/278, 279/280, 318/319, 319/320 and/or 339/340) in hJNK3-kinase or a peptide bond in proximity to these positions in hJNK3-kinase, or peptide bonds in protein targets related to hJNK3-kinase at positions having structural homology or sequence homology to these positions.

In a ninety-third embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human C-X-C chemokine receptor type 4 (hCCR-4). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, inflammation, as well as other diseases connected with hCCR-4. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hCCR-4 (SEQ ID NO: 189). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 7/8, 10/11, 12/13, 14/15, 15/16, 20/21, 21/22, 22/23, 25/26, 26/27, 30/31, 31/32, 32/33, 36/37, 38/39, 102/103, 103/104, 104/105, 107/108, 110/111, 181/182, 182/183, 183/184, 184/185, 187/188, 188/189, 190/191, 193/194, 195/196, 262/263, 268/269, 271/272, 275/276, 277/278, 282/283 and/or 283/284 in hCCR-4 or a peptide bond in proximity to these positions in

hCCR-4, or peptide bonds in protein targets related to hCCR-4 at positions having structural homology or sequence homology to these positions.

In a ninety-fourth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human beta-amyloid (hbeta-amyloid). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, Alzheimer, as well as other diseases connected with hbeta-amyloid. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hbeta-amyloid (SEQ ID NO: 190). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 1/2, 3/4, 5/6, 7/8, 10/11, 11/12, 16/17, 19/20, 20/21, 22/23, 23/24 and/or 28/29 (most preferred between positions 7/8, 10/11, 11/12, 16/17 and/or 23/24) in hbeta-amyloid or a peptide bond in proximity to these positions in hbeta-amyloid, or peptide bonds in protein targets related to hbeta-amyloid at positions having structural homology or sequence homology to these positions.

In a ninety-fifth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing human Tumor necrosis factor receptor superfamily member 14 (hvemA). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, inflammatory bowel disease, Crohn´s disease, colitis ulcerosa, as well as other diseases connected with hvemA. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hvemA (SEQ ID NO: 191). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 14/15, 18/19, 23/24, 24/25, 26/27, 31/32, 54/55, 62/63, 68/69, 71/72, 75/76, 95/96, 101/102 and/or 103/104 (most preferred between positions 23/24, 26/27, 62/63, 68/69, 95/96, 101/102 and/or 103/104) in hvemA or a peptide bond in proximity to these positions in hvemA, or peptide bonds in protein targets related to hvemA at positions having structural homology or sequence homology to these positions.

In a ninety-sixth embodiment of this variant the enzyme is a protease and is capable of hydrolyzing a TNF-superfamily member human B lymphocyte stimulator (hBlys). The enzymes or the fusion proteins can thus be used for preparing medicaments for the treatment of diseases, such as, but not limited to, acute or chronic inflammatory diseases like rheumatoid arthritis, inflammatory bowel diseases, psoriasis, Crohn's disease, Ulcerative colitis, Sjogren's syndrome,

systemic lupus erythematosus, multiple sclerosis, Systemic inflammatory response syndrome (SIRS), Graft-versus-host disease (GvhD) or sclerodermia, or for treatment of B-cell malignancies like classical Hodgkin's Lymphoma (cHL), mantle cell lymphoma, centrocytic lymphoma (CCL) or follicular lymphoma, as well as other diseases connected with hBlys. Preferably, said enzyme or said fusion protein is capable of specifically inactivating hBlys (SEQ ID NO: 192). More preferably said enzyme or said fusion protein is capable of hydrolysing the peptide bonds between positions 160/161, 163/164, 173/174, 174/175, 181/182, 188/189, 192/193, 204/205, 206/207, 214/215, 215/216, 216/217, 222/223, 231/232, 252/253, 257/258, 265/266 and/or 273/274 (most preferred between positions 160/161, 163/164, 181/182, 188/189 and/or 222/223) in hBlys or a peptide bond in proximity to these positions in hBlys, or peptide bonds in protein targets related to hBlys at positions having structural homology or sequence homology to these positions.

In some examples, the enzyme is a protease and is capable of hydrolyzing a target given in Table 1a to treat a pathology or disease associated with that protein.

It is obvious to someone skilled in the art that also polymorphisms of all target sequences referred to are included. The expression "proximity to these positions" in all embodiments above refer to positions of peptide bonds that are between 10 and 5 Ångström and/or 5 amino acids, preferably 3 amino acids, next to the positions of the peptide bonds.

Table 1a

	Target for NBE cleavage	Disease or condition to be improved
1	a5B1 (VLA-5)	cancer cell migration and adhesion of several cancers including lung cancers and myelomas
2	ADAM-12-S	Cancer
3	ADAM-9	Cancer
4	Adiponectin (also called GBP-28, apM1, AdipoQ and Acrp30)	chronic renal failure, type I diabetes, anorexia nervosa
5	ADP receptors (e..g, ADP receptor P2Y(12), ADP receptor P2T(AC), ADP receptor P2Y(1))	trombosis and platelet diseases
6	advanced glycation endproducts receptor (RAGE)	diabetes
7	Aldose reductase	diabetes, including autoimmune diabetes
8	angiotensin-converting enzyme (ACE)	diabetes mellitus
9	Anthrax: EF: Edema Factor	Anthrax
10	Anthrax: LF: Leathal Factor	Anthrax
11	Anthrax: PA, Protective Antigen	Anthrax

12	AP-1	intimal hyperplasia - vascular remodeling upon blood vessel injury
13	B7-1, B7-2, CD28	Graft-v.-host disorder, rheumatoid arthritis, transplant rejection, diabetes mellitus
14	BAD	apoptosis associated disorders, immunodeficiency diseases (AIDS/HIV), senescence, degenerative disorders (neurodegenerative diseases), ischemic and reperfusion cell death, acute ischemic injury, infertility, infectious colitis, inflammatory bowel disease (IBD), in particular in Crohn's disease, improved heart function after heart attack, cell death in Salmonella infections
15	BAX	apoptosis associated disorders, immunodeficiency diseases (AIDS/HIV), senescence, degenerative disorders (neurodegenerative diseases), ischemic and reperfusion cell death, acute ischemic injury, infertility, infectious colitis, inflammatory bowel disease (IBD), in particular in Crohn's disease, improved heart function after heart attack, cell death in Salmonella infections
16	Bcl-2	cancer
17	BCR-Abl	cancer
18	beta-catenin	cancer (e.g., metastasis)
19	beta-lactamases from bacteria (e.g., Pseudomonas aeruginosa, Moraxella (Branhamella) catarrhalis)	Infections including cystic fibrosis and chronic lung infection, pneumonia or bronchitis
20	BLyS	Systemic lupus erythematosus
21	Bovine, swine, sheep, human and other animal glycophosphatidylinositol (GPI)-anchored protein PrP(C) or their isoforms, PrP(Sc)	animal prion diseases
22	C5/C5a	Asthma ; Coronary artery bypass graft (CABG) surgery, Acute Pancreatitis, Inflamm.
23	Carbohydrate sulfotransferases (e.g., NodH sulfotransferase, UDP-glucuronosyltransferase, Heparan sulfate 3-o-sulfotransferase isoform 3, human estrogen sulfotransferase, phenol sulfotransferase SULT1A1 (ST1A3), human GalCer sulfotransferase)	inflammation, viral infection and cancer
24	caspase-6	apoptosis associated disorders, immunodeficiency diseases (AIDS/HIV), senescence, degenerative disorders (neurodegenerative diseases), ischemic and reperfusion cell death, acute ischemic injury, infertility, infectious colitis, inflammatory bowel disease (IBD), in particular in Crohn's disease, improved heart function after heart attack, cell death in Salmonella infections

25	Caspase-1 (IL1beta converting enzyme (ICE)	infectious colitis, inflammatory bowel disease (IBD), in particular in Crohn's disease, improved heart function after heart attack or ischaemia/reperfusion, cell death in Salmonella infections
26	caspase-8	Parkinson's disease.
27	CCR8	Asthma, COPD, Chr Bronchitis
28	CD18	inflammation
29	CD20	NHL
30	CD22	NHL
31	CD22	NHL
32	CD25, IL-2 receptor	Transplant rejection
33	CD3	Graft-versus-host disease, Transplant rejection and rejection prophylaxis; Type I diabetis,
34	CD30L receptor	Cancer, malignant lymphoma, classical Hodgkin's Lymphoma
35	CD30L	classical Hodgkin's Lymphoma (cHL)
36	CD33	AML, acute myelogenous leukemia
37	CD35	RA, Transplant rejection
38	CD4	HIV, psoriasis, transplant rejection and graft-versus-host colitis and autoimmune disorders; rheumatoid arthritis
39	CD40	classical Hodgkin's Lymphoma (cHL); Graft-v.-host disorder, transplant rejection, psoriasis
40	CD40L	Acute Pancreatitis, systemic lupus erythematosus, classical Hodgkin's Lymphoma (cHL)
41	CD46 (MCP)	renal tumors, uveal melanomas, gastrointestinal tumours and other forms of cancer
42	CD52	B-CLL
43	CD55 (DAF)	renal tumors, gastric and other forms of cancer
44	CD59	renal tumors and other forms of cancer
45	cdk-4	cancer
46	chitin from fungal pathogens	Fungal infections
47	CINC/GRO-alpha	Acute Pancreatitis, Inflammation
48	c-Jun	intimal hyperplasia - vascular remodeling upon blood vessel injury
49	ClfA	Staph. aureus infections
50	c-met (Hepatocyte growth factor receptor)	angiogenic growth factor in cancers
51	CO-029 or other human tetraspanin proteins	Cancer
52	Corticotropin-releasing hormone (CRH)	Modulation of arousal, modulation of reproductive behavior and function, Modulation of behavior in feeding, cerebrospinal hypercortisolemia, anxiety and affective disorders, melancholic depression as well as post-traumatic stress disorder (PTSD)
53	CTLA-4 (CD152)	Breast cancer
54	CXCR1	Asthma, COPD, Chr Bronchitis
55	CXCR2	Asthma, COPD, Chr Bronchitis
56	cyclo-oxygenase (COX)	thrombosis
57	cytocrome C	(same as caspase)
58	diacylglycerol acyltransferase (DGAT)	diet induced obesity and diabetes

59	ErbB3 (Her-3), ErbB4 (Her4)	cancer
60	EGFR endodomain (intracellular)	metastatic adenocarcinoma (cancer) of the colon or rectum or stage III colon cancer or metastatic epidermal growth factor receptor-positive colorectal cancer or Cancer of the Oropharynx, Hypopharynx, or Larynx or Head and Neck Cancer
61	eotaxin	classical Hodgkin's Lymphoma (cHL) and eosinophilia-mediated inflammation
62	EPA1 (e.g., from Candida glabrata and other fungal pathogens)	fungemia, mucosal infection
63	Ep-CAM (epithelial cell adhesion molecule) EGP-2	colorectal cancer
64	ERK	intimal hyperplasia - vascular remodeling upon blood vessel injury
65	E-Selectin	Prostate Cancer
66	exfoliative toxin (e.g., from Staphylococcus aureus)	scalded skin syndrome (SSS)
67	exorphins (e.g., gluten exorphin A5, B4, B5 and C; alpha-casein exorphin on CA1)	Autism and schizophrenia
68	F protein (e.g., from RSV)	RSV
69	factor Xa	trombosis including deep vein thrombosis
70	fibrinogen	cardiovascular disorders
71	G(q/11)	trombosis and G(q)-mediated diseases
72	gangliosides (GT3, GD3 and especially GM-1 and the islet-specific monosialo-ganglioside GM2-1)	autoimmune diabetes
73	glycogen phosphorylase (GP)	type 2 diabetes
74	GM-CSF	cystic fibrosis, Lung Inflammation, classical Hodgkin's Lymphoma (cHL) and eosinophilia-mediated inflammation
75	gp41	HIV infection
76	Hag (e.g., from Moraxella (Branhamella) catarrhalis)	pneumonia or bronchitis
77	hemaglutinin	influenza infection
78	Heme Oxygenase	CF, Lung Inflammation
79	HIF I	cancer
80	Histone deacetylase	Cancer
81	IgE/ IgER	Graft-v.-host disorder, transplant rejection
82	IGF	breast cancer
83	IL-12 / IL-12 receptor	Crohn's disease, inflammatory bowel disease, classical Hodgkin's Lymphoma (cHL), multiple sclerosis
84	IL-13R	asthma, fibrosis, psoriasis and atopic dermatitis, classical Hodgkin's Lymphoma (cHL).
85	IL-15/IL-15R	psoriasis, acute myeloid leukemia, rheumatoid arthritis, inflammation or inflammatory bowel disease and in diseases associated with the retrovirus HTLV-I (human T-cell lymphotropic virus I)

86	IL-18 receptor ("IL-1- related protein", IL-1Rp)	inflammation, organ and graft rejection
87	IL-27	asthma, inflammation, rheumatic disorders
88	IL-2R alpha and beta	autoimmune disorders, Graft-v.-host disorders, rheumatoid arthritis, T-cell leukemia/lymphoma
89	IL-31	asthma, inflammation, rheumatic disorders
90	IL-5R	asthma, classical Hodgkin's Lymphoma (cHL), and eosinophilia-mediated inflammation
91	IL-7	classical Hodgkin's Lymphoma (cHL)
92	IL-9	Chronic Obstructive Pulmonary Disease, classical Hodgkin's Lymphoma (cHL), airway inflammation and asthma
93	inner layer protein p24 (e.g. from HIV)	AIDS
94	Integrin a(4) b(1)	multiple sclerosis; Crohn's disease, inflammatory bowel disease
95	Integrin a(4) b(7)	Crohn's
96	Integrin a(v) b(3)	Melanoma
97	Integrin b(1)	Cron's disease, inflammatory bowel disease
98	Integrin b(7)	Cron's disease, inflammatory bowel disease
99	interleukin 11;	Cron's disease, inflammatory bowel disease
100	IP-10, Mig, MIP-1alpha	classical Hodgkin's Lymphoma (cHL)
101	IRAK-1	Inflammation, Sepsis, and Autoimmunity
102	IRAK-4	Inflammation, Sepsis, and Autoimmunity
103	Jun N-terminal kinase (JNK)	intimal hyperplasia - vascular remodeling upon blood vessel injury and diabetes
104	Kallikrein	hereditary angioedema (HAE)
105	leukocyte function-associated antigen-1	organ and graft rejection
106	leukotriene B(4)	Chronic Obstructive Pulmonary Disease, inflammation
107	leukotriene D_4 (LTD_4)	Chronic Obstructive Pulmonary Disease, inflammation
108	leukotriene receptor Cys-LT_1	Chronic Obstructive Pulmonary Disease, inflammation
109	leukotriene receptor Cys-LT_2	Chronic Obstructive Pulmonary Disease, inflammation
110	leukotriene receptor LTB4-1, LTB4-2	Chronic Obstructive Pulmonary Disease, inflammation
111	leukotriene receptors	Chronic Obstructive Pulmonary Disease, inflammation
112	Lewis y/b antigen	Cancer ; Lung cancer
113	lipoprotein(a)	cardiovascular disorders
114	LT-alpha	classical Hodgkin's Lymphoma (cHL)
115	lyphotoxin beta	colitis, diabetes, arthritis, infammation
116	matrix metalloprotease-1 (MMP-1)	emphysema
117	mcaP adherence protein (e.g., from Moraxella (Branhamella) catarrhalis)	pneumonia or bronchitis
118	MCP-1	Acute Pancreatitis, Inflamm.
119	M-CSF	classical Hodgkin's Lymphoma (cHL)
120	MDC	classical Hodgkin's Lymphoma (cHL) and eosinophilia-mediated inflammation

121	MHC class II receptors	lymphomas and other cancers including non-Hodgkin's lymphoma, Hodgkin's lymphoma, multiple myeloma and hairy cell leukemia.
122	MID (e.g., from Moraxella (Branhamella) catarrhalis)	pneumonia or bronchitis
123	MMP-12	emphysema
124	MMP-13	cancer
125	MN antigen	Liver cancer
126	muscarinic receptor, M1 and M3	Lung diseases, e.g., Chronic Obstructive Pulmonary Disease
127	NAD(P)H oxidase	vascular complications associated with diabetes and other diseases related to reactive oxygen species (ROS)
128	neutrophil elastase	Chronic Obstructive Pulmonary Disease
129	NF-kappaB	Chronic Obstructive Pulmonary Disease, atherosclerosis and thrombosis
130	nucleocapsid p17 (e.g., from HIV)	AIDS
131	p10 protease (e.g., from HIV)	AIDS
132	p115-RhoGEF A-site	cancer (e.g., metastasis)
133	p32 integrase (e.g., from HIV)	AIDS
134	p64 Reverse transcriptase (e.g., from HIV)	AIDS
135	PAF	Acute Pancreatitis, Inflamm.
136	parathyroid hormone	chronic renal failure, Cardiovascular disease
137	parathyroid hormone-related peptide (PTHrP) receptor	chronic renal failure, Cardiovascular disease
138	PDE3A, Platelet cyclic adenosine monophosphate (cAMP) phosphodiesterase	thrombosis
139	phosphodiesterase 4	Chronic Obstructive Pulmonary Disease
140	Polymorphic epithelial mucin (PEM) (MUC-1)	Cancer (Solid tumors), Ovarian cancer
141	porin F (OprF) (e.g., from Pseudomonas aeruginosa)	human alveolar epithelial adhesin
142	Proteasome subunits	trombosis including arterial thrombosis
143	Protein-Tyrosine Phosphatase PTPase 1B (PTP1B)	Diabetes and Related States of Insulin Resistance
144	PTH receptor	chronic renal failure, Cardiovascular disease
145	RANK	classical Hodgkin's Lymphoma (cHL)
146	RANKL	classical Hodgkin's Lymphoma (cHL)
147	Rip2	bacteria-induced inflammation
148	RSV (respiratory syncytium virus) fusion protein	RSV infection
149	Sortase (e.g., from Streptococcus mutans)	Caries
150	Src-Homology Inositol Phosphatase-2 (SHIP2)	type 2 diabetes mellitus.
151	T1/ST2	Inflammation, for example, eosinophilic inflammation of the airways
152	TARC	classical Hodgkin's Lymphoma (cHL) and eosinophilia-mediated inflammation

153	TGF beta-1, 2, 3, 4	Glaucoma, suppression of cell-mediated immunity
154	TGF-betaRI	diffuse systemic sclerosis
155	thrombin	blood clotting
156	tissue factor/factor VIIa	trombosis including venous thrombosis
157	Toll-like Receptors (TLRs) 1-10	CF, Lung Inflammation
158	transmembrane PTPase leukocyte antigen-related (LAR)	Diabetes and Related States of Insulin Resistance
159	triggering receptor expressed on myeloid cells (TREM)-1	CF, Lung Inflammation, septic shock, cancer, acute pancreatitis
160	UspA1(e.g., from Moraxella (Branhamella) catarrhalis)	pneumonia or bronchitis
161	VAP-1 (Vascular adhesion protein-1)	inflammation
162	VEGFR-3	cancer
163	Wnt proteins 2, 3, 4, and 7B	Cancer (e.g., breast cancer)
164	OSM receptor	inflammation, rheumatoid arthritis, inflammatory bowel disease
165	IL-6 receptor alpha chain	inflammation, rheumatoid arthritis, inflammatory bowel disease
166	IL-6 receptor beta chain	inflammation, rheumatoid arthritis, inflammatory bowel disease
167	lymphotoxin beta receptor	inflammation, rheumatoid arthritis, inflammatory bowel disease
168	leukemia inhibitory factor receptor	inflammation, rheumatoid arthritis, inflammatory bowel disease

Preferably, in this variant the scaffold of the engineered enzyme provided in step (c) is of human origin in order to avoid or reduce immunogenicity or allergenic effects associated with the application of the enzyme in the human body.

Alternatively, immunogenicity and allergenicity can be reduced by deimmunization of the engineered enzyme. Deimmunization in this context refers to the removal or exchange of those amino acid residues that confer immunogenicity or allergenicity to the engineered enzyme.

In further embodiment of this variant, the target substrate is a pro-drug which is activated by the engineered enzyme. In a particular embodiment of this variant, the engineered enzyme has proteolytic activity and the target substrate is a protein target which is proteolytically activated. Examples of such pro-drugs are pro-proteins such as the inactivated forms of coagulations factors. In another particular variant, the engineered enzyme is an oxidoreductase and the target substrate is a chemical that can be activated by oxidation.

In a second variant of this aspect of the invention, the engineered enzyme is used for diagnostic puposes. In a particular embodiment of this variant, the engineered enzyme is target-specific protease. Such diagnostic purposes comprise but are not

limited to applications with the aim of diagnosing diseases, testing genetic predispositions or monitoring disease progression during therapy. In a particular embodiment, the diagnosis is based on the testing for the presence or absence of a disease-specific marker protein or a disease-specific variant of a human protein in test samples such as human tissue samples, blood samples or other samples taken from patients. The testing employs a protease with specificity for a particular, disease-related target protein. The testing is done by analysing the proteolytic degradation of such protein in the test sample. In a preferred embodiment the aim of the diagnostic test is to detect and/or quantify a disease-specific variant of a native human protein. Such a diagnostic test employs a protease that is specific for the disease-related protein variant, i.e. it has significantly higher proteolytic activity on the disease-related protein variant compared to the native human protein. The disease-related protein variant is therefore detected and/or quantified by detecting and/or quantifying the activity of the target-specific protease. Such detection and/or quantification is done by directly measuring the degradation products of the target protein or indirectly by measuring the influence of the target protein on the activity of the target-specific protease by a competition assay. In another preferred embodiment the aim of the diagnostic test is to detect and/or quantify a protein that is specific for an infection by an infectious agent such as a virus or a bacterium. Such a diagnostic test employs a protease that is specific for a protein specifically expressed upon infection by the infectious agent, i.e. it has significantly higher proteolytic activity on a particular infection-indicating protein compared to any other native human protein. The infection-indicating protein is therefore detected and/or quantified by detecting and/or quantifying the proteolytic activity of the target-specific protease. Such detection and/or quantification is done by directly measuring the degradation products of the infection-indicating protein or indirectly by measuring the influence of the infection-indicating protein on the activity of the target-specific protease by a competition assay.

In a third variant of this aspect of the invention, the engineered enzyme is used as a technical means in order to catalyze an industrially or nutritionally relevant reaction with defined specificity. In a particular embodiment of this variant the engineered enzyme has proteolytic activity, the catalyzed reaction is a proteolytic processing, and the engineered enzyme specifically hydrolyses one or more industrially or nutrionally relevant protein substrates. In a preferred embodiment of

this variant the engineered enzyme hydrolyses one or more industrially or nutrionally relevant protein substrates at specific sites, thereby leading to industrially or nutrionally desired product properties such as texture, taste or precipitation characteristics. In a further particular embodiment of this variant, the engineered enzyme catalyzes the hydrolysis of glycosidic bonds (glycosidase or glycosylases activity). Then, preferably, the catalyzed reaction is a polysaccharide processing, and the engineered enzyme specifically hydrolyses one or more industrially, technically or nutrionally relevant polysaccharide substrates. In a further particular embodiment of this variant, the engineered enzyme catalyzes the hydrolysis of triglyceride esters or lipids (lipase activity). Then, preferably, the catalyzed reaction is a lipid processing step, and the engineered enzyme specifically hydrolyses one or more industrially, technically or nutrionally relevant lipid substrates. In a further particular variant of this embodiment, the engineered enzyme catalyzes the oxidation or reduction of substrates (oxidoreductase activity). Then, preferably, the engineered enzyme specifically oxidizes or reduces one or more industrially, technically or nutrionally relevant chemical substrates.

A second aspect of the invention discloses engineered enzymes with defined specificities. These engineered enzymes are characterized by the following components:

(a) a protein scaffold capable of catalyzing at least one chemical reaction on a substrate, and

(b) one or more specificity determining regions (SDRs) located at sites in the protein scaffold that enable the resulting engineered protein to discriminate between at least one target substrate and one or more different substrates, wherein the SDRs are essentially synthetic peptide sequences.

Preferably, such defined specificity of the engineered enzymes is not conferred by the protein scaffold.

In principle, the protein scaffold can have a variety of primary, secondary and tertiary structures. The primary structure, i.e. the amino acid sequence, can be an engineered sequence or can be derived from any viral, prokaryotic or eukaryotic origin. For human therapeutic use, however, the protein scaffold is preferably of mammalian origin, and more preferably, of human origin. Furthermore, the protein scaffold is capable to catalyze one or more chemical reactions and has preferably only a low specificity.

Preferably, derivatives of the protein scaffold are used that have modified amino acid sequences that confer improved characteristics for the applicability as protein scaffolds. Such improved characteristics comprise, but are not limited to, stability; expression or secretion yield; folding, in particular after combination of the protein scaffold with SDRs; increased or decreased sensitivity to regulators such as activators or inhibitors; immunogenicity; catalytic rate; kM or substrate affinity.

The engineered enzymes reveal their quantitative specificity from the peptide sequences that are combined with the protein scaffold. Therefore, the engineered peptide sequences are acting as Specificity Determining Regions or SDRs. The number, the length and the positions of such SDRs can vary over a wide range. The number of SDRs within the scaffold is at least one, preferably more than one, more preferably between two and eleven, most preferably between two and six. The SDRs have a length between one and 50 amino acid residues, preferably a length between one and 15 amino acid residues, more preferably a length between one and six amino acid residues. Alternatively, the SDRs have a length between two and 20 amino acid residues, preferably a length between two and ten amino acid residues, more preferably a length between three and eight amino acid residues.

The inventive engineered enzymes can further be desribed as antibody-like protein molecules comprising constant and variable regions, but having a non-immunoglogulin backbone and having an active site (catalytic activity) in the constant region, whereby the substrate specificity of the active site is modulated by the variable region. Preferably, as in the immunoglobulin structure, the variable regions are loops of variable length and composition that interact with a target molecule.

In a particular variant of the invention, the engineered enzymes have hydrolase activity. In a preferred variant, the engineered enzymes have proteolytic activity. Particularly preferred protein scaffolds for this variant are unspecific proteases or are parts from unspecific proteases or are otherwise derived from unspecific proteases. The expressions "derived from" or "a derivative thereof" in this respect and in the following variants and embodiments refer to derivatives of proteins that are mutated at one or more amino acid positions and/or have a homology of at least 70%, preferably 90%, more preferably 95% and most preferably 99% to the original protein, and/or that are proteolytically processed, and/or that have an altered glycosylation pattern, and/or that are covalently linked to non-protein substances, and/or that are fused with further protein domains, and/or that have

C-terminal and/or N-terminal truncations, and/or that have specific insertions, substitutions and/or deletions. Alternatively, "derived from" may refer to derivatives that are combinations or chimeras of two or more fragments from two or more proteins, each of which optionally comprises any or all of the aforementioned modifications. The tertiary structure of the protein scaffold can be of any type. Preferably, however, the tertiary structure belongs to one of the following structural classes: class S1 (chymotrypsin fold of the serine proteases family), class S8 (subtilisin fold of the serine proteases family), class SC (carboxypeptidase fold of the serine proteases family), class A1 (pepsin A fold of the aspartic proteases), or class C14 (caspase-1 fold of the cysteine proteases). Examples of proteases that can serve as the protein scaffold of engineered proteolytic enzymes for the use as human therapeutics are or are derived from human trypsin, human thrombin, human chymotrypsin, human pepsin, human endothiapepsin, human caspases 1 to 14, and/or human furin.

The defined specificity of the engineered proteolytic enzymes is a measure of their ability to discriminate between at least one target peptide or protein substrates and one or more further peptide or protein substrates. Preferably, the defined specificity refers to the ability to discriminate peptide or protein substrates that differ in other positions than the P1 site, more preferably, the defined specificity refers to the ability to discriminate peptide or protein substrates that differ in other positions than the P1 site and the P1' site. Most preferably, the engineered proteolytic enzymes distinguish target peptid or protein substrates at as many sites as is necessary to preferentially hydrolyse the target substrate versus other proteins. As an example, a therapeutically useful engineered proteolytic enzyme applied intravenously in the human body should be sufficiently specific to discriminate between the target substrate and any other protein in the human serum. Preferably, such an engineered proteolytic enzyme recognizes and discriminates peptide substrates at three or more amino acid positions, more preferably at four or more positions, and even more preferably at five or more amino acid positions. These positions may either be adjacent or non-adjacent.

In a first embodiment , the protein scaffold has a tertiary structure or fold equal or similar to the tertiary structure or fold of the S1 structural subclass of serine proteases, i. e. the chymotrypsin fold, and/or has at least 70% identity on the amino acid level to a protein of the S1 structural subclass of serine proteases. It is preferred that SDRs are inserted into the protein scaffold at one or more positions

from the group of positions that correspond structurally or by amino acid sequence homology to the regions 18-25, 38-48, 54-63, 73-86, 122-130, 148-156, 165-171 and 194-204 in human trypsin I, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 20-23, 41-45, 57-60, 76-83, 125-128, 150-153, 167-169 and 197-201 (numbering of amino acids according to SEQ ID NO:1). The number of SDRs to be combined with this type of protein scaffold is preferably between 1 and 10, and more preferably between 2 and 4. Preferably, the protein scaffold is equal to or is a derivative or homologue of one or more of the following proteins: chymotrypsin, granzyme, kallikrein, trypsin, mesotrypsin, neutrophil elastase, pancreatic elastase, enteropeptidase, cathepsin, thrombin, ancrod, coagulation factor IXa, coagulation factor VIIa, coagulation factor Xa, activated protein C, urokinase, tissue-type plasminogen activator, plasmin, Desmodus-type plasminogen activator. More preferably, the protein scaffold is trypsin or thrombin or is a derivative or homologue from trypsin or thrombin. For the use as a human therapeutic, the trypsin or thrombin scaffold is most preferably of human origin in order to minimize the risk of an immune response or an allergenic reaction.

Preferably, derivatives with improved characteristics derived from human trypsin I or from proteins with similar tertiary structure are used. Preferred examples of such derivatives are derived from human trypsin I (SEQ ID NO:1) and comprise one or more of the following amino acid substitutions E56G; R78W; Y131F; A146T; C183R.

It is preferred that at least one of two SDRs are inserted into human trypsin I, or a derivative thereof, between residues 42 and 43 (SDR 1) and between 123 and 124 (SDR 2), respectively (numbering of amino acids according to SEQ ID NO:1). In addition the SDR 1 has a preferred length of 6 and the SDR 2 has a preferred length of 5 amino acids, respectively. In a preferred variant of this embodiment, the SDR 1 and SDR 2 sequences comprise one of the amino acid sequences listed in table 2. Such engineered proteolytic enzymes have specificity for the target substrate B as exemplified in example IV.

In a further embodiment the protein scaffold belongs to the S8 structural subclass of serine proteases and/or has a tertiary structure similar to subtilisin E from Bacillus subtilis_and/or has at least 70% identity on the amino acid level to a protein of the S8 structural subclass of serine proteases. Preferably, the scaffold belongs to the subtilisin family or the human pro-protein convertases. It is preferred that SDRs are inserted into the protein scaffold at one or more positions

from the group of positions that correspond structurally or by amino acid sequence homology to the regions 6-17, 25-29, 47-55, 59-69, 101-111, 117-125, 129-137, 139-154, 158-169, 185-195 and 204-225 in subtilisin E from Bacillus subtilis, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 59-69, 101-111, 129-137, 158-169 and 204-225 (numbering of amino acids according to SEQ ID NO:7). It is preferred that the protein scaffold is equal to or is a derivative or homologue of one or more of the following proteins: subtilisin Carlsberg; B. subtilis subtilisin E; subtilisin BPN'; B. licheniformis subtilisin; B. lentus subtilisin; Bacillus alcalophilus alkaline protease; proteinase K; kexin; human pro-protein convertase; human furin. In a preferred variant, subtilisin BPN' or one of the proteins SPC 1 to 7 is used as the protein scaffold.

In a further embodiment the protein scaffold belongs to the family of aspartic proteases and/or has a tertiary structure similar to human pepsin. Preferably, the scaffold belongs to the A1 class of proteases and/or has at least 70% identity on the amino acid level to a protein of the A1 class of proteases. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 6-18, 49-55, 74-83, 91-97, 112-120, 126-137, 159-164, 184-194, 242-247, 262-267 and 277-300 in human pepsin, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 10-15, 75-80, 114-118, 130-134, 186-191 and 280-296 (numbering of amino acids according to SEQ ID NO:11). It is preferred that the protein scaffold is equal to or is a derivative or homologue of one or more of the following proteins: pepsin, chymosin, renin, cathepsin, yapsin. Preferably, pepsin or endothiopepsin or a derivative or homologue thereof is used as the protein scaffold.

In a further embodiment the protein scaffold belongs to the cysteine protease family and/or has a tertiary structure similar to human caspase 7. Preferably the scaffold belongs to the C14 class of cysteine proteases or has at least 70% identity on the amino acid level to a protein of the C14 class of cysteine proteases. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 78-91, 144-160, 186-198, 226-243 and 271-291 in human caspase 7, and more preferably at one or more positions from the group of

positions that correspond structurally or by amino acid sequence homology to the regions 80-86, 149-157, 190-194 and 233-238 (numbering of amino acids according to SEQ ID NO:14). It is preferred that the protein scaffold is equal to or is a derivative or homologue of one of the caspases 1 to 9.

In a further embodiment the protein scaffold belongs to the S11 class of serine proteases or has at least 70% identity on the amino acid level to a protein of the S11 class of serine proteases and/or has a tertiary structure similar to D-alanyl-D-alanine transpeptidase from Streptomyces species K15. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 67-79, 137-150, 191-206, 212-222 and 241-251 in D-alanyl-D-alanine transpeptidase from Streptomyces species K15, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 70-75, 141-147, 195-202 and 216-220 (numbering of amino acids according to SEQ ID NO:15). It is preferred that the D-alanyl-D-alanine transpeptidase from Streptomyces species K15 or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the S21 class of serine proteases or has at least 70% identity on the amino acid level to a protein of the S21 class of serine proteases and/or has a tertiary structure similar to assemblin from human cytomegalovirus. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 25-33, 64-69, 134-155, 162-169 and 217-244 in assemblin from human cytomegalovirus, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 27-31, 164-168 and 222-239 (numbering of amino acids according to SEQ ID NO:16). It is preferred that the assemblin from human cytomegalovirus or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the S26 class of serine proteases or has at least 70% identity on the amino acid level to a protein of the S26 class of serine proteases and/or has a tertiary structure similar to the signal peptidase from Escherichia coli. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 8-14,

57-68, 125-134, 239-254, 200-211 and 228-239 in signal peptidase from Escherichia coli, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 9-13, 60-67, 127-132 and 203-209 (numbering of amino acids according to SEQ ID NO:17). It is preferred that the signal peptidase from Escherichia coli or a derivative or homologue thereof is used as the scaffold.

In an further embodiment the protein scaffold belongs to the S33 class of serine proteases or has at least 70% identity on the amino acid level to a protein of the S33 class of serine proteases and/or has a tertiary structure similar to the prolyl aminopeptidase from Serratia marcescens. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 47-54, 152-160, 203-212 and 297-302 in prolyl aminopeptidase from Serratia marcescens, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 50-53, 154-158 and 206-210 (numbering of amino acids according to SEQ ID NO:18). It is preferred that the prolyl aminopeptidase from Serratia marcescens or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the S51 class of serine proteases or has at least 70% identity on the amino acid level to a protein of the S51 class of serine proteases and/or has a tertiary structure similar to aspartyl dipeptidase from Escherichia coli. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 8-16, 38-46, 85-92, 132-140, 159-170 and 205-211 in aspartyl dipeptidase from Escherichia coli, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 10-14, 87-90, 134-138 and 160-165 (numbering of amino acids according to SEQ ID NO:19). It is preferred that the aspartyl dipeptidase from Escherichia coli or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the A2 class of aspartic proteases or has at least 70% identity on the amino acid level to a protein of the A2 class of aspartic proteases and/or has a tertiary structure similar to the protease from human immunodeficiency virus. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that

correspond structurally or by amino acid sequence homology to the regions 5-12, 17-23, 27-30, 33-38 and 77-83 in protease from human immunodeficiency virus, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 7-10, 18-21, 34-37 and 79-82 (numbering of amino acids according to SEQ ID NO:20). It is preferred that the protease from human immunodeficiency virus, preferably HIV-1 protease, or a derivative or homologue thereof is used as the scaffold.

In an further embodiment the protein scaffold belongs to the A26 class of aspartic proteases or has at least 70% identity on the amino acid level to a protein of the A26 class of aspartic proteases and/or has a tertiary structure similar to the omptin from Escherichia coli. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 28-40, 86-98, 150-168, 213-219 and 267-278 in omptin from Escherichia coli, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 33-38, 161-168 and 273-277 (numbering of amino acids according to SEQ ID NO:21). It is preferred that the omptin from Escherichia coli or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the C1 class of cysteine proteases or has at least 70% identity on the amino acid level to a protein of the C1 class of cysteine proteases and/or has a tertiary structure similar to the papain from Carica papaya. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 17-24, 61-68, 88-95, 135-142, 153-158 and 176-184 in papain from Carica papaya, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 63-66, 136-139 and 177-181 (numbering of amino acids according to SEQ ID NO:22). It is preferred that the papain from Carica papaya or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the C2 class of cysteine proteases or has at least 70% identity on the amino acid level to a protein of the C2 class of cysteine proteases and/or has a tertiary structure similar to human calpain-2. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 90-103, 160-172, 193-199, 243-260, 286-

294 and 316-322 in human calpain-2, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 92-101, 245-250 and 287-291 (numbering of amino acids according to SEQ ID NO:23). It is preferred that the human calpain-2 or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the C4 class of cysteine proteases or has at least 70% identity on the amino acid level to a protein of the C4 class of cysteine proteases and/or has a tertiary structure similar to NIa protease from tobacco etch virus. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 23-31, 112-120, 144-150, 168-176 and 205-218 in NIa protease from tobacco etch virus, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 145-149, 169-174 and 212-218 (numbering of amino acids according to SEQ ID NO:24). It is preferred that the NIa protease from tobacco etch virus (TEV protease) or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the C10 class of cysteine proteases or has at least 70% identity on the amino acid level to a protein of the C10 class of cysteine proteases and/or has a tertiary structure similar to the streptopain from Streptococcus pyogenes. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 81-90, 133-140, 150-164, 191-199, 219-229, 246-256, 306-312 and 330-337 in streptopain from Streptococcus pyogenes, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 82-87, 134-138, 250-254 and 331-335 (numbering of amino acids according to SEQ ID NO:25). It is preferred that the streptopain from Streptococcus pyogenes or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the C19 class of cysteine proteases or has at least 70% identity on the amino acid level to a protein of the C19 class of cysteine proteases and/or has a tertiary structure similar to human ubiquitin specific protease 7. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond

structurally or by amino acid sequence homology to the regions 3-15, 63-70, 80-86, 248-256, 272-283 and 292-304 in human ubiquitin specific protease 7, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 10-15, 251-255, 277-281 and 298-304 (numbering of amino acids according to SEQ ID NO:26). It is preferred that the human ubiquitin specific protease 7 or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the C47 class of cysteine proteases or has at least 70% identity on the amino acid level to a protein of the C47 class of cysteine proteases and/or has a tertiary structure similar to the staphopain from Staphylococcus aureus. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 15-23, 57-66, 108-119, 142-149 and 157-164 in staphopain from Staphylococcus aureus, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 17-22, 111-117, 143-147 and 159-163 (numbering of amino acids according to SEQ ID NO:27). It is preferred that the staphopain from Staphylococcus aureus or a derivative or homologue thereof is used as the scaffold.

In an further embodiment the protein scaffold belongs to the C48 class of cysteine proteases or has at least 70% identity on the amino acid level to a protein of the C48 class of cysteine proteases and/or has a tertiary structure similar to the Ulp1 endopeptidase from Saccharomyces cerevisiae. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 40-51, 108-115, 132-141, 173-179 and 597-605 in Ulp1 endopeptidase from Saccharomyces cerevisiae, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 43-49, 110-113, 133-137 and 175-178 (numbering of amino acids according to SEQ ID NO:28). It is preferred that the Ulp1 endopeptidase from Saccharomyces cerevisiae or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the C56 class of cysteine proteases or has at least 70% identity on the amino acid level to a protein of the C56 class of cysteine proteases and/or has a tertiary structure similar to the Pfpl

endopeptidase from Pyrococcus horikoshii. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 8-16, 40-47, 66-73, 118-125 and 147-153 in Pfpl endopeptidase from Pyrococcus horikoshii, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 9-14, 68-71, 120-123 and 148-151 (numbering of amino acids according to SEQ ID NO:29). It is preferred that the Pfpl endopeptidase from Pyrococcus horikoshii or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the M4 class of metallo proteases or has at least 70% identity on the amino acid level to a protein of the M4 class of metallo proteases and/or has a tertiary structure similar to thermolysin from Bacillus thermoproteolyticus. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 106-118, 125-130, 152-160, 197-204, 210-213 and 221-229 in thermolysin from Bacillus thermoproteolyticus, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 108-115, 126-129, 199-203 and 223-227 (numbering of amino acids according to SEQ ID NO:30). It is preferred that the thermolysin from Bacillus thermoproteolyticus or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the M10 class of metallo proteases or has at least 70% identity on the amino acid level to a protein of the M10 class of metallo proteases and/or has a tertiary structure similar to human collagenase. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 2-7, 68-79, 85-90, 107-111 and 135-141 in human collagenase, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 3-6, 71-78 and 136-140 (numbering of amino acids according to SEQ ID NO:31). It is preferred that human collagenase or a derivative or homologue thereof is used as the scaffold.

It is further preferred that the engineered enzymes have glycosidase activity. A particularly suited protein scaffold for this variant is a glycosylase or is derived from

a glycosylase. Preferably, the tertiary structure belongs to one of the following structural classes: class GH13, GH7, GH12, GH11, GH10, GH28, GH26, and GH18 (beta/alpha)8 barrel.

In a first embodiment the protein scaffold belongs to the GH13 class of glycosylases or has at least 70% identity on the amino acid level to a protein of the GH13 class of glycosylases and/or has a tertiary structure similar to human pancreatic alpha-amylase. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 50-60, 100-110, 148-167, 235-244, 302-310 and 346-359 in human pancreatic alpha-amylase, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 51-58, 148-155 and 303-309 (numbering of amino acids according to SEQ ID NO:32). It is preferred that human pancreatic alpha-amylase or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the GH7 class of glycosylases or has at least 70% identity on the amino acid level to a protein of the GH7 class of glycosylases and/or has a tertiary structure similar to cellulase from Trichoderma reesei. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 47-56, 93-104, 173-182, 215-223, 229-236 and 322-334 in cellulase from Trichoderma reesei, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 175-180, 218-222 and 324-332 (numbering of amino acids according to SEQ ID NO:33). It is preferred that cellulase from Trichoderma reesei or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the GH12 class of glycosylases or has at least 70% identity on the amino acid level to a protein of the GH12 class of glycosylases and/or has a tertiary structure similar to cellulase from Aspergillus niger. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 18-28, 55-60, 106-113, 126-132 and 149-159 in cellulase from Aspergillus niger, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid

sequence homology to the regions 20-26, 56-59, 108-112 and 151-156 (numbering of amino acids according to SEQ ID NO:34). It is preferred that cellulase from Aspergillus niger or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the GH11 class of glycosylases or has at least 70% identity on the amino acid level to a protein of the GH11 class of glycosylases and/or has a tertiary structure similar to xylanase from Aspergillus niger. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 7-14, 33-39, 88-97, 114-126 and 158-167 in xylanase from Aspergillus niger, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 20-26, 56-59, 108-112 and 151-156 (numbering of amino acids according to SEQ ID NO:35). It is preferred that xylanase from Aspergillus niger or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the GH10 class of glycosylases or has at least 70% identity on the amino acid level to a protein of the GH10 class of glycosylases and/or has a tertiary structure similar to xylanase from Streptomyces lividans. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 21-29, 42-50, 84-92, 130-136, 206-217 and 269-278 in xylanase from Streptomyces lividans, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 43-49, 86-90, 208-213 and 271-276 (numbering of amino acids according to SEQ ID NO:36). It is preferred that xylanase from Streptomyces lividans or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the GH28 class of glycosylases or has at least 70% identity on the amino acid level to a protein of the GH28 class of glycosylases and/or has a tertiary structure similar to pectinase from Aspergillus niger. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 82-88, 118-126, 171-178, 228-236, 256-264 and 289-299 in pectinase from Aspergillus niger, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 116-124, 174-178 and 291-296

(numbering of amino acids according to SEQ ID NO:37). It is preferred that pectinase from Aspergillus niger or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the GH26 class of glycosylases or has at least 70% identity on the amino acid level to a protein of the GH26 class of glycosylases and/or has a tertiary structure similar to mannanase from Pseudomonas cellulosa. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 75-83, 113-125, 174-182, 217-224, 247-254, 324-332 and 325-340 in mannanase from Pseudomonas cellulosa, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 115-123, 176-180, 286-291 and 328-337 (numbering of amino acids according to SEQ ID NO:38). It is preferred that mannanase from Pseudomonas cellulosa or a derivative or homologue thereof is used as the scaffold.

In an further embodiment the protein scaffold belongs to the GH18 (beta/alpha)8 barrel class of glycosylases or has at least 70% identity on the amino acid level to a protein of the GH18 class of glycosylases and/or has a tertiary structure similar to chitinase from Bacillus circulans. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 21-29, 57-65, 130-136, 176-183, 221-229, 249-257 and 327-337 in chitinase from Bacillus circulans, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 59-63, 178-181, 250-254 and 330-336 (numbering of amino acids according to SEQ ID NO:39). It is preferred that chitinase from Bacillus circulans or a derivative or homologue thereof is used as the scaffold.

It is further preferred that the engineered enzymes have esterhydrolase activity. Preferably, the protein scaffold for this variant have lipase, phosphatase, phytase, or phosphodiesterase activity.

In a first embodiment the protein scaffold belongs to the GX class of esterases or has at least 70% identity on the amino acid level to a protein of the GX class of esterases and/or has a tertiary structure similar to the structure of the lipase B from Candida antarctica. Preferably, the scaffold has lipase activity. It is preferred

that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 139-148, 188-195, 216-224, 256-266, 272-287 in lipase B from Candida antarctica, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 141-146, 218-222, 259-263 and 275-283 (numbering of amino acids according to SEQ ID NO:40). It is preferred that lipase B from Candida antarctica or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the GX class of esterases or has at least 70% identity on the amino acid level to a protein of the GX class of esterases and/or has a tertiary structure similar to the pancreatic lipase from guinea pig. Preferably, the scaffold has lipase activity. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 78-90, 91-100, 112-120, 179-186, 207-218, 238-247 and 248-260 in pancreatic lipase from guinea pig, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 80-87, 114-118, 209-215 and 239-246 (numbering of amino acids according to SEQ ID NO:41). It is preferred that pancreatic lipase from guinea pig or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold has a tertiary structure similar to the structure of the alkaline phosphatase from Escherichia coli or has at least 70% identity on the amino acid level to a protein that has a tertiary structure similar to the structure of the alkaline phosphatase from Escherichia coli. Preferably, the scaffold has phosphatase activity. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 110-122, 187-142, 170-175, 186-193, 280-287 and 425-435 in alkaline phosphatase from Escherichia coli, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 171-174, 187-191, 282-286 and 426-433 (numbering of amino acids according to SEQ ID NO:42). It is preferred that alkaline phosphatase from Escherichia coli or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold has a tertiary structure similar to the structure of the bovine pancreatic desoxyribonuclease I or has at least 70% identity

on the amino acid level to a protein that has a tertiary structure similar to the structure of the bovine pancreatic desoxyribonuclease I. Preferably, the scaffold has phosphodiesterase activity. More preferably, a nuclease, and most preferably, an unspecific endonuclease or a derivative thereof is used as the scaffold. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 14-21, 41-47, 72-77, 97-111, 135-143, 171-178, 202-209 and 242-251 in bovine pancreatic desoxyribonuclease I, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 16-19, 42-46, 136-141 and 172-176 (numbering of amino acids according to SEQ ID NO:43). It is preferred that bovine pancreatic desoxyribonuclease I or human desoxyribonuclease I or a derivative or homologue thereof is used as the scaffold.

It is further preferred that the engineered enzyme has transferase activity. A particularly suited protein scaffold for this variant is a glycosyl-, a phospho- or a methyltransferase, or is a derivative thereof. Particularly preferred protein scaffolds for this variant are glycosyltransferases or are derived from glycosyltransferases. The tertiary structure of the protein scaffold can be of any type. Preferably, however, the tertiary structure belongs to one of the following structural classes: GH13 and GT1.

In a first embodiment the protein scaffold belongs to the GH13 class of transferases or has at least 70% identity on the amino acid level to a protein of the GH13 class of transferases and/or has a tertiary structure similar to the structure of the cyclomaltodextrin glucanotransferase from Bacillus circulans. Preferably, the scaffold has transferase activity, and more preferably a glycosyltransferase is used as the scaffold. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 38-48, 85-94, 142-154, 178-186, 259-266, 331-340 and 367-377 in cyclomaltodextrin glucanotransferase from Bacillus circulans, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 87-92, 180-185, 261-264 and 269-275 (numbering of amino acids according to SEQ ID NO:44). It is preferred that cyclomaltodextrin glucanotransferase from Bacillus circulans or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold belongs to the GT1 class of tranferases or has at least 70% identity on the amino acid level to a protein of the GT1 class of transferases and/or has a tertiary structure similar to the structure of the glycosyltransferase from Amycolatopsis orientalis A82846. Preferably the scaffold has transferase activity, and more preferably glycosyltransferase activity. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 58-74, 130-138, 185-193, 228-236 and 314-323 in glycosyltransferase from Amycolatopsis orientalis A82846, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 61-71, 230-234 and 316-321 (numbering of amino acids according to SEQ ID NO:45). It is preferred that the glycosyltransferase from Amycolatopsis orientalis A82846 or a derivative or homologue thereof is used as the scaffold.

It is further preferred that the engineered enzymes have oxidoreductase activity. A particularly suited protein scaffold for this variant is a monooxygenase, a dioxygenase or a alcohol dehydrogenase, or a derivative thereof. The tertiary structure of the protein scaffold can be of any type.

In a first embodiment the protein scaffold has a tertiary structure similar to the structure of the 2,3-diphydroxybiphenyl dioxygenase from Pseudomonas sp. or has at least 70% identity on the amino acid level to a protein that has a tertiary structure similar to the structure of the 2,3-diphydroxybiphenyl dioxygenase from Pseudomonas sp. Preferably, the scaffold has dioxygenase activity. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 172-185, 198-206, 231-237, 250-259 and 282-287 in 2,3-diphydroxybiphenyl dioxygenase from Pseudomonas sp., and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 175-182, 200-204, 252-257 and 284-287 (numbering of amino acids according to SEQ ID NO:46). It is preferred that the 2,3-diphydroxybiphenyl dioxygenase from Pseudomonas sp or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold has a tertiary structure similar to the structure of the catechol dioxygenase from Acinetobacter sp. or has at least 70% identity on the amino acid level to a protein that has a tertiary structure similar to

the structure of the catechol dioxygenase from Acinetobacter sp.. Preferably, the scaffold has dioxygenase activity, and more preferably catechol dioxygenase activity. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 66-72, 105-112, 156-171 and 198-207 in catechol dioxygenase from Acinetobacter sp., and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 107-110, 161-171 and 201-205 (numbering of amino acids according to SEQ ID NO:47). It is preferred that the catechol dioxygenase from Acinetobacter sp or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold has a tertiary structure similar to the structure of the camphor-5-monooxygenase from Pseudomonas putida or has at least 70% identity on the amino acid level to a protein that has a tertiary structure similar to the structure of the camphor-5-monooxygenase from Pseudomonas putida. Preferably, the scaffold has monooxygenase activity, and more preferably camphor monooxygenase activity. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 26-31, 57-63, 84-98, 182-191, 242-256, 292-299 and 392-399 in camphor-5-monooxygenase from Pseudomonas putida, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 85-96, 183-188, 244-253, 293-298 and 393-398 (numbering of amino acids according to SEQ ID NO:48). It is preferred that the camphor-5-monooxygenase from Pseudomonas putida or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold has a tertiary structure similar to the structure of the alcohol dehydrogenase from Equus callabus or has at least 70% identity on the amino acid level to a protein that has a tertiary structure similar to the structure of the alcohol dehydrogenase from Equus callabus. Preferably, the scaffold has alcohol dehydrogenase activity. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 49-63, 111-112, 294-301 and 361-369 in alcohol dehydrogenase from Equus callabus, and more preferably at one or more positions from the group of positions that

correspond structurally or by amino acid sequence homology to the regions 51-61 and 295-299 (numbering of amino acids according to SEQ ID NO:49). It is preferred that the alcohol dehydrogenase from Equus callabus or a derivative or homologue thereof is used as the scaffold.

It is further preferred that the engineered enzymes have lyase activity. A particularly suited protein scaffold for this variant is a oxoacid lyase or is a derivative thereof. Particularly preferred protein scaffolds for this variant are aldolases or synthases, or are derived thereof. The tertiary structure of the protein scaffold can be of any type, but a (beta/alpha)8 barrel structure is preferred.

In a first embodiment the protein scaffold has a tertiary structure similar to the structure of the N-acetyl-d-neuramic acid aldolase from Escherichia coli or has at least 70% identity on the amino acid level to a protein that has a tertiary structure similar to the structure of the N-acetyl-d-neuramic acid aldolase from Escherichia coli. Preferably, the scaffold has aldolase activity. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 45-55, 78-87, 105-113, 137-146, 164-171, 187-193, 205-210, 244-255 and 269-276 in N-acetyl-d-neuramic acid aldolase from Escherichia coli, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 45-52, 138-144, 189-192, 247-253 and 271-275 (numbering of amino acids according to SEQ ID NO:50). It is preferred that the N-acetyl-d-neuramic acid aldolase from Escherichia coli or a derivative or homologue thereof is used as the scaffold.

In a further embodiment the protein scaffold has a tertiary structure similar to the structure of the tryptophan synthase from Salmonella typhimurium or has at least 70% identity on the amino acid level to a protein that has a tertiary structure similar to the structure of the tryptophan synthase from Salmonella typhimurium. Preferably, the scaffold has synthase activity. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 56-63, 127-134, 154-161, 175-193, 209-216 and 230-240 in tryptophan synthase from Salmonella typhimurium, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 57-62, 155-160, 178-190 and 210-215 (numbering of amino acids according to SEQ ID NO:51). It is preferred that the tryptophan synthase from

Salmonella typhimurium or a derivative or homologue thereof is used as the scaffold.

It is further preferred that the engineered enzymes have isomerase activity. A particularly suited protein scaffold for this variant is a converting aldose or a converting ketose, or is a derivative thereof.

In a first embodiment, the protein scaffold has a tertiary structure similar to the structure of the xylose isomerase from Actinoplanes missouriensis or has at least 70% identity on the amino acid level to a protein that has a tertiary structure similar to the structure of the xylose isomerase from Actinoplanes missouriensis. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 18-31, 92-103, 136-147, 178-188 and 250-257 in xylose isomerase from Actinoplanes missouriensis, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 20-27, 92-99 and 180-186 (numbering of amino acids according to SEQ ID NO:52). It is preferred that the xylose isomerase from Actinoplanes missouriensis or a derivative or homologue thereof is used as the scaffold.

It is further preferred that the engineered enzymes have ligase activity. A particularly suited protein scaffold for this variant is a DNA ligase, or is a derivative thereof.

In a first embodiment, the protein scaffold has a tertiary structure similar to the structure of the DNA ligase from Bacteriophage T7 or has at least 70% identity on the amino acid level to a protein that has a tertiary structure similar to the structure of the DNA-ligase from Bacteriophage T7. It is preferred that SDRs are inserted into the protein scaffold at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 52-60, 94-108, 119-131, 241-248, 255-263 and 302-318 in DNA ligase from Bacteriophage T7, and more preferably at one or more positions from the group of positions that correspond structurally or by amino acid sequence homology to the regions 96-106, 121-129, 256-262 and 304-316 (numbering of amino acids according to SEQ ID NO:53). It is preferred that the DNA ligase from Bacteriophage T7 or a derivative or homologue thereof is used as the scaffold.

A third aspect of the invention is directed to a method for generating engineered enzymes with specificities that are qualitatively and/or quantitatively novel in combination with the protein scaffold. The inventive method comprises at least the following steps:

(a) providing a protein scaffold capable to catalyze at least one chemical reaction on at least one target substrate,

(b) generating a library of engineered enzymes or isolated engineered enzymes by combining the protein scaffold from step (a) with one or more fully or partially random peptide sequences at sites in the protein scaffold that enable the resulting engineered enzyme to discriminate between at least one target substrate and one or more different substrates and

(c) selecting out of the library of engineered enzymes generated in step (b) one or more enzymes that have defined specificities towards at least one target substrate.

In a first variant of this aspect of the invention, the inventive method comprises at least the following steps:

(a) providing a protein scaffold capable to catalyze at least one chemical reaction on at least one target substrate,

(b) generating a library of engineered enzymes or isolated engineered enzymes by inserting into the protein scaffold from step (a) one or more fully or partially random peptide sequences at sites in the protein scaffold that enable the resulting engineered enzyme to discriminate between at least one target substrate and one or more different substrates and

(c) selecting out of the library of engineered enzymes generated in step (b) one or more enzymes that have defined specificities towards at least one target substrate.

Preferably, the positions at which the one or more fully or partially random peptide sequences are combined with or inserted into the protein scaffold are identified prior to the combination or insertion.

The number of insertions or other combinations of fully or partially random peptide sequences as well as their length may vary over a wide range. The number is at least one, preferably more than one, more preferably between two and eleven, most preferably between two and six. The length of such fully or partially random peptide sequences is usually less than 50 amino acid residues. Preferably, the length is between one and 15 amino acid residues, more preferably between one

and six amino acid residues. Alternatively, the length is between two and 20 amino acid residues, preferably between two and ten amino acid residues, more preferably between three and eight amino acid residues.

Preferably such insertions or other combinations are performed on the DNA level, using polynucleotides encoding such protein scaffolds and polynucleotides or oligonucleotides encoding such fully or partially random peptide sequences.

Optionally, steps (a) to (c) are repeated cyclically, whereby enzymes selected in step (c) serve as the protein scaffold in step (a) of a further cycle, and randomized peptide sequences are either inserted or, alternatively, substituted for peptide sequences that have been inserted in former cycles. Thereby, the number of inserted peptide sequences is either constant or increases over the cycles. The cycles are repeated until one or more enzymes with the intended specificities are generated.

Moreover, during or after one or more rounds of steps (a) to (c), the scaffold may be mutated at one or more positions in order to make the scaffold more acceptable for the combination with SDR sequences, and/or to increase catalytic activity at a specific pH and temperature, and/or to change the glycosylation pattern, and/or to decrease sensitivity towards enzyme inhibitors, and/or to change enzyme stability.

In a second variant of this aspect of the invention, the inventive method comprises at least the following steps:
(a) providing a first protein scaffold fragment,
(b) connecting said protein scaffold fragment via a peptide linkage with a first SDR, and optionally
(c) connecting the product of step (b) via a peptide linkage with a further SDR peptide or with a further protein scaffold fragment, and optionally
(d) repeating step (c) for as many cycles as necessary in order to generate a sufficiently specific enzyme, and
(e) selecting out of the population generated in steps (a) – (d) one or more enzymes that have the desired specificities toward the one or more target substrates.

Protein scaffold fragment means a part of the sequence of a protein scaffold. A protein scaffold is comprised of at least two protein scaffold fragments.

In a third variant of this aspect of the invention, the protein scaffold, the SDRs and the engineered enzyme are encoded by a DNA sequence and an expression system

is used in order to produce the protein. In an alternative variant, the protein scaffold, the SDRs and/or the engineered enzyme are chemically synthesized from peptide building blocks.

In a fourth variant of this aspect of the invention, the inventive method comprises at least the following steps:
(a) providing a polynucleotide encoding a protein scaffold capable of catalyzing one or more chemical reactions on one or more target substrates;
(b) combining one or more fully or partially random oligonucleotide sequence with the polynucleotide encoding the protein scaffold, the fully or partially random oligonucleotide sequences being located at sites in the polynucleotide that enable the encoded engineered enzyme to discriminate between the one or more target substrates and one or more other substrates; and
(c) selecting out of the population generated in step (b) one or more polynucleotides that encode enzymes that have the defined specificities toward the one or more target substrates.

Any enzyme can serve as the protein scaffold in step (a). It can be a naturally occurring enzyme, a variant or a truncated derivate therefore, or an engineered enzyme. For human therapeutic use, the protein scaffold is preferably a mammalian enzyme, and more preferably a human enzyme. In that aspect, the invention is directed to a method for the generation of essentially mammalian, especially of essentially human enzymes with specificities that are different from specificities of any enzyme encoded in mammalian genomes or in the human genome, respectively.

According to the invention, the protein scaffold provided in step (a) of this aspect requires to be capable of catalyzing one or more chemical reactions on a target substrate. Therefore, a protein scaffold is selected from the group of potential protein scaffolds by its activity on the target substrate.

In a preferred variant of this aspect of the invention, a protein scaffold with hydrolase activity is used. Preferably, a protein scaffold with proteolytic activity is used, and more preferably, a protease with very low specificity having basic activity on the target substrate is used as the protein scaffold. Examples of proteases from different structural classes with low substrate specificity are Papain, Trypsin, Chymotrypsin, Subtilisin, SET (trypsin-like serine protease from Streptomyces erythraeus), Elastase, Cathepsin G or Chymase. Before being employed as the

protein scaffold, the amino acid sequence of the protease may be modified in order to change protein properties other than specificity, e.g catalytic activity, stability, inhibitor sensitivity, or expression yield, essentially as described in WO 92/18645, or in order to change specificity, essentially as described in EP 02020576.3 and PCT/EP03/04864.

Another option for a feasible protein scaffold are lipases. Hepatic lipase, lipoprotein lipase and pancreatic lipase belong to the "lipoprotein lipase superfamily", which in turn is an example of the GX-class of lipases (M. Fischer, J. Pleiss (2003), Nucl. Acid. Res., 31, 319-321). The substrate specificity of lipases can be characterized by their relative activity towards triglycerol esters of fatty acids and phospholipids, bearing a charged head group. Alternatively, other hydrolases such as esterases, glycosylases, amidases, or nitrilases may be used as scaffolds.

Transferases are also feasible protein scaffolds. Glycoslytransferases are involved in many biological synthesis involving a variety of donors and acceptors. Alternatively, the protein scaffold may have ligase, lyase, oxidoreductase, or isomerase activity.

In a first embodiment, the one or more fully or partially random peptide sequences are inserted at specific sites in the protein scaffold. These insertion sites are characterized by the fact that the inserted peptide sequences can act as discriminators between different substrates, i.e. as Specificity Determining Regions or SDRs. Such insertion sites can be identified by several approaches. Preferably, insertion sites are identified by analysis of the three-dimensional structure of the protein scaffolds, by comparative analysis of the primary sequences of the protein scaffold with other enzymes having different quantitative specificities, or experimentally by techniques such as alanine scanning, random mutagenesis, or random deletion, or by any combination thereof.

A first approach to identify insertion sites for SDRs bases on the three-dimensional structure of the protein scaffold as it can be obtained by x-ray crystallography or by nuclear magnetic resonance studies. Structural alignment of the protein scaffold in comparison with other enzymes of the same structural class but having different quantitative specificities reveals regions of high structural similarity and regions with low structural similarity. Such an analysis can for example be done using public software such as Swiss PDB viewer (Guex, N. and Peitsch, M.C. (1997)

Electrophoresis 18, 2714-2723). Regions of low structural similarity are preferred SDR insertion sites.

In a second approach to identify insertion sites for SDRs, three-dimensional structures of the scaffold protein in complex with competitive inhibitors or substrate analogs are analysed. It is assumed that the binding site of a competitive inhibitor significantly overlaps with the binding site of the substrate. In that case, atoms of the protein that are within a certain distance of atoms of the inhibitor are likely to be in a similar distance to the substrate as well. Choosing a short distance, e.g. < 5 Å, will result in an ensemble of protein atoms that are in close contact with the substrate. These residues would constitute the first shell contacts and are therefore preferred insertion sites for SDRs. Once first shell contacts have been identified, second shell contacts can be found by repeating the distance analysis starting from first shell atoms. In yet another alternative of the invention the distance analysis described above is performed starting from the active site residues.

In third approach to identify insertion sites for SDRs, the primary sequence of the scaffold protein is aligned with other enzymes of the same structural class but having different quantitative specificities using an alignment algorithm. Examples of such alignment algorithms are published (Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) J. Mol. Biol. 215:403-410; "Statistical methods in Bioinformatics: an introduction" by Ewens, W. & Grant, G.R. 2001, Springer, New York). Such an alignment may reveal conserved and non-conserved regions with varying sequence homology, and, in particular, additional sequence elements in one or more enzymes compared to the scaffold protein. Conserved regions of are more likely to contribute to phenotypes shared among the different proteins, e.g. stabilizing the three-dimensional fold. Non-conserved regions and, in particular, additional sequences in enzymes with quantitatively higher specificity (Turner, R. et al. (2002) *J. Biol. Chem.*, 277, 33068-33074) are preferred insertion sites for SDRs.

For proteases currently five families are known, namely aspartic-, cysteine-, serine-, metallo- and threonine proteases. Each family includes groups of proteases that share a similar fold. Crystallographic structures of members of these groups have been solved and are accessible through public databases, e.g. the Brookhaven protein database (H.M. Berman et al. *Nucleic Acids Research*, 28 pp. 235-242 (2000)). Such databases also include structural homologs in other enzyme classes and nonenzymatically active proteins of each class. Several tools are available to search public databases for structural homologues: SCOP - a structural

classification of proteins database for the investigation of sequences and structures. (Murzin A. G. et al. (1995) *J. Mol. Biol.* 247, 536-540); CATH - Class, Architecture, Topology and Homologous superfamily: a hierarchical classification of protein domain structures (Orengo et al. (1997) *Structure* 5(8) 1093-1108); FSSP - Fold classification based on structure-structure alignment of proteins (Holm and Sander (1998) *Nucl. Acids Res.* 26 316-319); or VAST - Vector alignment search tool (Gibrat, Madej and Bryant (1996) *Current Opinion in Structural Biology* 6, 377-385).

In the above described approaches, members of structural classes are compared in order to identify insertion sites for SDRs.

In a preferred variant of these approaches serine proteases of the structural class S1 are compared with each other. Trypsin represents a member with low substrate specificity, as it requires only an arginine or lysine residue at the P_1 position. On the other hand, thrombin, tissue-type plasminogen activator or enterokinase all have a high specificity towards their substrate sequences, i.e. (L/I/V/F)XPR^ NA, CPGR^ VVGG and DDDK^ , respectively (Perona, J. & Craik, C. (1997) *J. Biol. Chem.*, 272, 29987-29990; Perona, J. & Craik, C (1995) *Protein Science*, 4, 337-360). An alignment of the amino acid sequences of these proteases is described in example 1 (Figure 2) along with the identification of SDRs.

A further example within the family of serine proteases is given by members of the structural class S8 (subtilisin fold). Subtilisin is the type protease for this class and represents an unspecific protease (Ottesen,M. & Svendsen,A. (1998) *Methods Enzymol.* 19, 199-215). Furin, PC1 and PC5 are proteases of the same structural class involved in the processing of propeptides and have a high substrate specificity (Seidah, N. & Chretien, M. (1997) Curr. Opin. Biotech., 8: 602-607; Bergeron, F. et al. (2000) *J. Mol. Endocrin.*, 24:1-22). In a preferred variant of the approach alignments of the primary amino acids sequences (Figure 4) are used to identify eleven sequence stretches longer than three amino acids which specific proteases have in addition compared to subtilisin and are therefore potential specificity determining regions. In a further variant of the approach information from the three-dimensional structure of subtilisin can be used in order to further narrow down the selection (Figure 3). Out of the eleven inserted sequence stretches, three are especially close to the active site residues, namely stretch number 7, 8 and 11 which are insertions in PC5, PC1 and all three specific proteases, respectively (Figure 3). In a preferred variant, one or several amino acid stretches of variable

length and composition can be inserted into the subtilisin sequence at one or several of the eleven positions. In a more preferred variant of the approach the insertion is performed at regions 7, 8 or 11 or any combination thereof. In another preferred variant of the approach protease scaffolds other than subtilisin from the structural class S8 are used.

In a further preferred variant of this approach, aspartic acid proteases of the structural class A1 are analyzed (Rawlings, N.D. & Barrett, A.J. (1995). *Methods Enzymol.* 248, 105-120; Chitpinityol, S. & Crabbe, MJ. (1998), *Food Chemistry*, 61, 395-418). Examples for the A1 structural class of aspartic proteases are pepsin with a low as well as beta-secretase (Grüninger-Leitch, F., et al. (2002) *J. Biol. Chem.* 277, 4687-4693) and renin (Wang, W. & Liang, TC. (1994) *Biochemistry*, 33, 14636-14641) with relatively high substrate specificities. Retroviral proteases also belong to this class, although the active enzyme is a dimer of two identical subunits. The viral proteases are essential for the correct processing of the polyprotein precursor to generate functional proteins which requires a high substrate specificity in each case (Wu, J. et al. (1998) *Biochemistry*, 37, 4518-4526; Pettit, S. et al. (1991) J. Biol. Chem., 266, 14539-14547). Pepsin is the type protease for this class and represents an unspecific protease (Kageyama, T. (2002) *Cell. Mol. Life Sci.* 59, 288-306). B-secretase and Cathepsin D (Aguilar, C. F. et al. (1995) *Adv. Exp. Med. Biol.* 362, 155-166) are proteases of the same structural class and have a high substrate specificity. In a preferred variant of the approach alignments of the primary amino acids sequences (Figure 6) are used to identify six sequence stretches longer than three amino acids which are inserted in the specific proteases compared to pepsin and are therefore potential specificity determining regions. In a further variant of the approach information from the three-dimensional structure of b-secretase can be used in order to further narrow down the selection. Out of the six inserted sequence stretches, three are especially close to the active site residues, namely stretch number 1, 3 and 4 which are insertions in cathepsin D and beta-secretase, respectively (Figure 5). In a preferred variant of the approach, one or several amino acid stretches of variable length and composition can be inserted into the pepsin sequence at one or several of the six positions. In a more preferred embodiment of the invention the insertion is performed at the positions 1, 3 or 4 or any combination thereof. In another preferred embodiment of the invention protease scaffolds other than pepsin are used.

There are cases where a certain structural class does not include known members of low and high specificity. This is exemplified by the C14 class of caspases which belong to the cysteine protease family (Rawlings, N.D. & Barrett, A.J. (1994) *Methods Enzymol.* 244, 461-486) and which all show high specificity for P_4 to P_1 positions. For example, caspase-1, caspase-3 and caspase-9 recognize the sequences YVAD^, DEVD^ or LEHD^, respectively. Identification of the regions that differ between the caspases will include the regions responsible for the differences in substrate specificity (Figures 7 and 8).

Finally, non-enzymatic proteins of the same fold as the enzyme scaffold may also contribute to the identification of insertion sites for SDRs. For example, haptoglobin (Arcoleo, J. & Greer, J.; (1982) J *Biol. Chem*. 257, 10063-10068) and azurocidin (Almeida, R. et al. (1991) Biochem. Biophys. Res. Commun. 177, 688-695) share the same chymotrypsin-like fold with all S1 proteases. Due to substitutions in the active site residues these proteins do not posses any proteolytic function, yet they show high homology with active proteases. Differences between these proteins and specific proteases include regions that can serve as insertion sites for SDRs.

In a fourth approach, insertion sites for SDRs are identified experimentally by techniques such as alanine scanning, random mutagenesis, random insertion or random deletion. In contrast to the approach disclosed above, this approach does not require detailed knowledge about the three-dimensional structure of the scaffold protein. In one preferred variant of this approach, random mutagenesis of enzymes with relatively high specificity from the same structural class as the protein scaffold and screening for loss or change of specificity can be used to identify insertion sites for SDRs in the protein scaffold.

Random mutagenesis, alanine scanning, random insertion or random deletion are all done on the level of the polynucleotides encoding the enzymes. There are a variety of protocols known in the literature (e.g. Sambrook, J.F; Fritsch, E.F.; Maniatis,T.; Cold Spring Harbor Laboratory Press, Second Edition, 1989, New York). For example, random mutagenesis can be achieved by the use of a polymerase as described in patent WO 9218645. According to this patent, the one or more genes encoding the one or more proteases are amplified by use of a DNA polymerase with a high error rate or under conditions that increase the rate of misincorporations. For example the method of Cadwell and Joyce can be employed (Cadwell, R.C. and Joyce, G.F., PCR methods. Appl. 2 (1992) 28-33). Other methods of random

mutagenesis such as, but not limited to, the use of mutator stains, chemical mutagens or UV-radiation can be employed as well.

Alternatively, oligonucleotides can be used for mutagenesis that substitute randomly distributed amino acid residues with an alanine. This method is generally referred to as alanine scanning mutagenesis (Fersht, A.R. Biochemistry (1989) 8031-8036). As a further alternative, modifications of the alanine scanning mutagenesis such as binominal mutagenesis (Gregoret, L.M. and Sauer, R.T. PNAS (1993) 4246-4250) or combinatorial alanine scanning (Weiss et al., PNAS (2000) 8950-8954) can be employed.

In order to express engineered enzymes, the DNA encoding such engineered proteins is ligated into a suitable expression vector by standard molecular cloning techniques (e.g. Sambrook, J.F; Fritsch, E.F.; Maniatis, T.; Cold Spring Harbor Laboratory Press, Second Edition, 1989, New York). The vector is introduced in a suitable expression host cell, which expresses the corresponding engineered enzyme variant. Particularly suitable expression hosts are bacterial expression hosts such as Escherichia coli or Bacillus subtilis, or yeast expression hosts such as Saccharomyces cerevisae or Pichia pastoris, or mammalian expression hosts such as Chinese Hamster Ovary (CHO) or Baby Hamster Kidney (BHK) cell lines, or viral expression systems such as bacteriophages like M13 or Lambda, or viruses such as the Baculovirus expression system. As a further alternative, systems for *in vitro* protein expression can be used. Typically, the DNA is ligated into an expression vector behind a suitable signal sequence that leads to secretion of the enzyme variants into the extracellular space, thereby allowing direct detection of protease activity in the cell supernatant. Particularly suitable signal sequences for Escherichia coli are HlyA, for Bacillus subtilis AprE, NprB, Mpr, AmyA, AmyE, Blac, SacB, and for S. cerevisiae Bar1, Suc2, Matα, Inu1A, Ggplp. Alternatively, the enzyme variants are expressed intracellularly and the substrates are expressed also intracellularly. Preferably, this is done essentially as described in patent application WO 0212543, using a fusion peptide substrate comprising two auto-fluorescent proteins linked by the substrate amino-acid sequence. As a further alternative, after intracellular expression of the enzyme variants, or secretion into the periplasmatic space using signal sequences such as DsbA, PhoA, PelB, OmpA, OmpT or gIII for Escherichia coli, a permeabilisation or lysis step releases the enzyme variants into the supernatant. The destruction of the membrane barrier can be forced by the use of mechanical means such as ultrasonic, French press, or the use of membrane-

digesting enzymes such as lysozyme. As another, further alternative, the genes encoding the enzyme variants are expressed cell-free by the use of a suitable cell-free expression system. For example, the S30 extract from Escherichia coli cells is used for this purpose as described by Lesly et al. (Methods in Molecular Biology 37 (1995) 265-278).

The ensemble of gene variants generated and expressed by any of the above methods are analyzed with respect to their affinity, substrate specificity or activity by appropriate assay and screening methods as described in detail for example in patent application PCT/EP03/04864. Genes from catalytically active variants having reduced specificity in comparison to the original enzyme are analyzed by sequencing. Sites at which mutations and/or insertions and/or deletions occurred are preferred insertion sites at which SDRs can be inserted site-specifically.

In a second embodiment, the one or more fully or partially random peptide sequences are inserted at random sites in the protein scaffold. This modification is usually done on the polynucleotide level, i.e. by inserting nucleotide sequences into the gene that encodes the protein scaffold. Several methods are available that enable the random insertion of nucleotide sequences. Systems that can be used for random insertion are for example ligation based systems (Murakami et al. Nature Biotechnology 20 (2002) 76-81), systems based on DNA polymerisation and transposon based systems (e.g. GPS-M™ mutagenesis system, NEB Biolabs; MGS™ mutation generation system, Finnzymes). The transposon-based methods employ a transposase-mediated insertion of a selectable marker gene that contains at its termini recognition sequences for the transposase as well as two sites for a rare cutting restriction endonuclease. Using the latter endonuclease one usually releases the selection marker and after religation obtains an insertion. Instead of performing the religation one can alternatively insert a fragment that has terminal recognition sequences for one or two outside cutting restriction endonuclease as well as a selectable marker. After ligation, one releases this fragment using the one or two outside cutting endonucleases. After creating blunt ends by standard methods one inserts blunt ended random fragments at random positions into the gene.

In a further preferred embodiment, methods for homologous in-vitro recombination are used to combine the mutations introduced by the above mentioned methods to generate enzyme populations. Examples of methods that can be applied are the Recombination Chain Reaction (RCR) according to patent application WO 0134835, the DNA-Shuffling method according to the patent application WO 9522625, the

Staggered Extension method according to patent WO 9842728, or the Random Priming recombination according to patent application WO9842728. Furthermore, also methods for non-homologous recombination such as the Itchy method can be applied (Ostermeier, M. et al. Nature Biotechnology 17 (1999) 1205-1209).

Upon random insertion of a nucleotide sequence into the protein scaffold one obtains a library of different genes encoding enzyme variants. The polynucleotide library is subsequently transferred to an appropriate expression vector. Upon expression in a suitable host or by use of an in vitro expression system, a library of enzymes containing randomly inserted stretches of amino acids is obtained.

According to step (b) of this third aspect of the invention, one or more fully or partially random peptide sequences are inserted into the protein scaffold. The actual number of such inserted SDRs is determined by the intended quantitative specificity following the relation: the higher the intended specificity is, the more SDRs are inserted. Whereas a single SDR enables the generation of moderately specific enzymes, two SDRs enable already the generation of significantly specific enzymes. However, up to six and more SDRs can be inserted into a protein scaffold. A similar relation is valid for the length of the SDRs: the higher the intended specificity is, the longer are the SDRs that are to be inserted. SDRs can be as short as one to four amino acid residues. They can, however, also be as long as 50 amino acid residues. Significant specificity can already be generated by the use of SDRs of a length of four to six amino acid residues.

The peptid sequences that are inserted can be fully or partially random. In this context, fully random means that a set of sequences are inserted in parallel that includes sequences that differ from each other in each and every position. Partially random means that a set of sequences are inserted in parallel that includes sequences that differ from each other in at least one position. This difference can be either pair-wise or with respect to a single sequence. For example, when regarding an insertion of the length of four amino acids, partial random could be a set (i) that includes AGGG, GVGG, GGLG, GGGI, or (ii) that includes AGGG, VGGG, LGGG and IGGG. Alternatively, random sequences also comprises sequences that differ from each other in length. Randomization of the peptide sequences is achieved by randomization of the nucleotide sequences that are inserted into the gene at the respective sites. Thereby, randomization can be achieved by employing mixtures of nucleobases as monomers during chemical synthesis of the oligonucleotides. A particularly preferred mixture of monomers for a fully random

codon that in addition minimizes the probability of stop codons is NN(GTC). Alternatively, random oligonucleotides can be obtained by fragmentation of DNA into short fragments that are inserted into the gene at the respective sites. The source of the DNA to be fragmented may be a synthetic oligonucleotide but alternatively may originate from cloned genes, cDNAs, or genomic DNA. Preferably, the DNA is a gene encoding an enzyme. The fragmentation can, for example, be achieved by random endonucleolytic digestion of DNA. Preferably, an unspecific endonuclease such as DNAse I (e.g. from bovine pancreas) is employed for the endonucleolytic digestion.

If steps (a) – (c) of the inventive method are repeated cyclically, there are different alternatives for obtaining random peptide sequences that are inserted in consecutive rounds. Preferably, SDRs that were identified in one round as leading to increased specificity of enzyme are used as templates for the random peptide sequences that are inserted in the following round.

In a preferred alternative, the sequences selected in one round are analysed and randomized oligonucleotides are generated based on these sequences. This can, for example, be achieved by using in addition to the original nucleotide with a certain percentage mixtures of the other three nucleotides monomers at each position in the oligonucleotide synthesis. If, for example, in a first round an SDRs is identified that has the amino acid sequence ARLT, e.g. encoded by the nucleotide sequence GCG CGC CTT ACC, a random peptide sequence inserted in this SDR site could be encoded by an oligonucleotide with 70% G, 10% A, 10% T and 10% C at the first position, 70% C, 10% G, 10% T and 10% A at the second position, etc. This leads at each position approximately in 1 of 3 cases to the template amino acid and in 2 of 3 cases to another amino acid.

In another preferred alternative, the sequences selected in one round are analyzed and a consensus library is generated based on these sequences. This can, for example, be achieved by using defined mixtures of nucleotides at each position in the oligonucleotide synthesis in a way that leads to mixtures of the amino acid residues that were identified at each position of the SDR selected in the previous round. If, for example, in a first round two SDRs are identified that have the amino acid sequences ARLT and VPGS, a consensus library inserted in this SDR site in the following round could be encoded by an oligonucleotide with the sequence G(C/T)G C(G/C)C (G/T)(G/T)G (A/T)CC. This would correspond to the random peptide sequence (A/V)(R/P)(L/G/V/W)(T/S), thereby allowing all combinations of the

amino acid residues identified in the first round, and, due to the degeneracy of the genetic code, allowing in addition to a lower degree alternative amino acid residues at some positions.

In another preferred alternative, the sequences selected in one round are, without previous analysis, recombined using methods for the in vitro recombination of polynucleotides, such as the methods described in WO 01/34835 (the following also provides details of the eighth and ninth aspect of the invention).

After insertion of the partially or fully random sequences into the gene encoding the scaffold protein, and eventually ligation of the resulting gene into a suitable expression vector using standard molecular cloning techniques (Sambrook, J.F; Fritsch, E.F.; Maniatis,T.; Cold Spring Harbor Laboratory Press, Second Edition, 1989, New York), the vector is introduced in a suitable expression host cell which expresses the corresponding enzyme variant. Particularly suitable expression hosts are bacterial expression hosts such as Escherichia coli or Bacillus subtilis, or yeast expression hosts such as Saccharomyces cerevisae or Pichia pastoris, or mammalian expression hosts such as Chinese Hamster Ovary (CHO) or Baby Hamster Kidney (BHK) cell lines, or viral expression systems such as bacteriophages like M13 T7 phage or Lambda, or viruses such as the Baculovirus expression system. As a further alternative, systems for *in vitro* protein expression can be used. Typically, the DNA is ligated into an expression vector behind a suitable signal sequence that leads to secretion of the enzyme variants into the extracellular space, thereby allowing direct detection of enzyme activity in the cell supernatant. Particularly suitable signal sequences for Escherichia coli are ompA, pelB, HlyA, for Bacillus subtilis AprE, NprB, Mpr, AmyA, AmyE, Blac, SacB, and for S. cerevisiae Bar1, Suc2, Matα, Inu1A, Ggplp. Alternatively, the enzyme variants are expressed intracellularly and the substrates are expressed also intracellularly. According to protease variants this is done essentially as described in patent application WO 0212543, using a fusion peptide substrate comprising two auto-fluorescent proteins linked by the substrate amino-acid sequence. As a further alternative, after intracellular expression of the enzyme variants, or secretion into the periplasmatic space using signal sequences such as DsbA, PhoA, PelB, OmpA, OmpT or gIII for Escherichia coli, a permeabilisation or lysis step releases the enzyme variants into the supernatant. The destruction of the membrane barrier can be forced by the use of mechanical means such as ultrasonic, French press, or the use of membrane-digesting enzymes such as lysozyme. As another, further

alternative, the genes encoding the enzyme variants are expressed cell-free by the use of a suitable cell-free expression system. For example, the S30 extract from Escherichia coli cells is used for this purpose as described by Lesly et al. (Methods in Molecular Biology 37 (1995) 265-278).

After introduction of the vector into host cells, these cells are screened for the expression of enzymes with specificity for the intended target substrate. Such screening is typically done by separating the cells from each other, in order to enable the correlation of genotype and phenotype, and assaying the activity of each cell clone after a growth and expression period. Such separation can for example be done by distribution of the cells into the compartments of sample carriers, e.g. as described in WO 01/24933. Alternatively, the cells are separated by streaking on agar plates, by enclosing in a polymer such as agarose, by filling into capillaries, or by similar methods.

Identification of variants with the intended specificity can be done by different approaches. In the case of proteases, preferably assays using peptide substrates essentially as described in PCT/EP03/04864 are employed.

Regardless of the expression format, selection of enzyme variants is done under conditions that allow identification of enzymes that recognize and convert the target sequence preferably. As a first alternative, enzymes that recognize and convert the target sequence preferably are identified by screening for enzymes with a high affinity for the target substrate sequence. High affinity corresponds to a low K_M which is selected by screening at target substrate concentrations substantially below the K_M of the first enzyme. Preferably, the substrates that are used are linked to one or more fluorophores that enable the detection of the modification of the substrate at concentrations below 10 µM, preferably below 1 µM, more preferably below 100 nM, and most preferably below 10 nM.

As a second alternative, enzymes that recognize and convert the target substrate preferably are identified by employing two or more substrates in the assay and screening for activity on these two or more substrates in comparison. Preferably, the two or more substrates employed are linked to different marker molecules, thereby enabling the detection of the modification of the two or more substrates consecutively or in parallel. In the case of proteases, particularly preferably two peptide substrates are employed, one peptide substrate having an arbitrarily chosen or even partially or fully random amino-acid sequence thereby enabling to

monitor the activity on an arbitrary substrate, and the other peptide substrate having an amino-acid sequence identical to or resembling the intended target substrate sequence thereby enabling to monitor the activity on the target substrate. Especially preferably, these two peptide substrates are linked to fluorescent marker molecules, and the fluorescent properties of the two peptide substrates are sufficiently different in order to distinguish both activities when measured consecutively or in parallel. For example, a fusion protein comprising a first autofluorescent protein, a peptide, and a second autofluorescent protein according to patent application WO 0212543 can be used for this purpose. Alternatively, fluorophores such as rhodamines are linked chemically to the peptide substrates.

As a third alternative, enzymes that recognize and convert the target substrate preferably are identified by employing one or more substrates resembling the target substrate together with competing substrates in high excess. Screening with respect to activity on the substrates resembling the target substrate is then done in the presence of the competing substrates. Enzymes having a specificity which corresponds qualitatively to the target specificity, but having only a low quantitative specificity are identified as negative samples in such a screen. Whereas enzymes having a specificity which corresponds qualitatively and quantitatively to the target specificity are identified positively. Preferably, the one or more substrates resembling the target substrate are linked to marker molecules, thereby enabling the detection of their modifications, whereas the competing substrates do not carry marker molecules. The competing substrates have arbitrarily chosen or random amino-acid sequences, thereby acting as competitive inhibitors for the hydrolysis of the marker-carrying substrates. For example, protein hydrolysates such as Trypton can serve as competing substrates for engineered proteolytic enzymes according to the invention.

As a fourth alternative, enzymes that recognize and convert the target substrate preferably are identified and selected by an amplification-coupled or growth-coupled selection step. Furthermore, the activity can be measured intracellularily and the selection can be done by a cell sorter, such as a fluorescence-activated cell sorter.

As a further alternative, enzymes that recognize and convert the target substrate are identified by first selecting enzymes that preferentially bind to the target substrate, and secondly selecting out of this subgroup of enzyme variants those

enzymes that convert the target substrate. Selection for enzymes that preferentially bind the target substrate can be either done by selection of binders to the target substrate or by counter-selection of enzymes that bind to other substrates. Methods for the selection of binders or for the counter-selection of non-binders is known in the art. Such methods typically require phenotype-genotype coupling which can be solved by using surface display expression methods. Such methods include, for example, phage or viral display, cell surface display and in vitro display. Phage or viral display typically involves fusion of the protein of interest to a viral/phage protein. Cell surface display, i.e. either bacterial or eukaryotic cell display, typically involves fusion of the protein of interest to a peptide or protein that is located at the cell surface. In in-vitro display, the protein is typically made in vitro and linked directly or indirectly to the mRNA encoding the protein (DE 19646372).

The invention also provides for a composition or pharmaceutical composition comprising one or more engineered enzymes according to the first aspect of the invention as defined herein before. The composition may optionally comprise an acceptable carrier, excipient and/or auxiliary agent.

Pharmaceutical compositions according to the invention may optionally comprise a pharmaceutically acceptable carrier. Pharmaceutical formulations are well known and pharmaceutical compositions may be routinely formulated by one having ordinary skill in the art. The composition can be formulated as a solution, suspension, emulsion, or lyophilized powderin association with a pharma-ceutically acceptable vehicle. Examples of such vehicles are water, saline, Ringer´s solution, dextrose solution, and human serum albumin. Liposomes and nonaaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e.g. sodium chloride, mannitol) and chemical stability (e.g. buffers and preservatives). The composition is sterilized by commonly used techniques.

The pharmaceutical composition of the present invention may be administrated by any means that enables the active agent to reach the agent´s site of action in the body of a mammal. Pharmaceutical compositions may be administered parentally, i.e. intravenous (i.v.), subcutaneous (s.c.), intramuscular.

Dosage varies depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration ;

age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired.

Non-pharmaceutical compositions as defined herein are research composition, nutritional composition, cleaning composition, desinfection composition, cosmetic composition or composition for personal care. Moreover, DNA sequences coding for the engineered enzyme as defined herein before and vectors containing said DNA sequences are also provided. Finally, transformed host cells (prokaryotic or eukaryotic) or transgenic organisms containing such DNA sequences and/or vectors, as well as a method utilizing such host cells or transgenic animals for producing the engineered enzyme of the first aspect of the invention are also contemplated.

Detailed description of the figures

Figure 1: Three-dimensional structure of human trypsin I with the active site residues shown in "ball-and-stick" representation and with the marked regions indicating potential SDR insertion sites.

Figure 2: Alignment of the primary amino acid sequences of the human proteases trypsin I, alpha-thrombin and enteropeptidase all of which belong to the structural class S1 of the serine protease family. Trypsin represents an unspecific protease of this structural class, while alpha-thrombin and enteropeptidase are proteases with high substrate specificity. Compared to trypsin several regions of insertions of three or more amino acids into the primary sequence of a-thrombin and enterokinase are seen. The region marked with (-1-) and the region marked with (-3-) are preferred SDR insertion sites. In the tertiary structure of alpha-thrombin both regions are in the vicinity of the substrate binding site. These regions therefore fullfil two criteria to be selected as candidates for SDRs: firstly, they represent insertions in the specific proteases compared to the unspecific one and, secondly, they are close to the substrate binding site. A representation of the three-dimensional structure is given in figure 3.

Figure 3: Three-dimensional structure of subtilisin with the active site residues being shown in "ball-and-stick" representation and with the numbered regions indicating potential SDR insertion sites.

Figure 4: Alignment of the primary amino acid sequences of subtilisin E, furin, PC1 and PC5 all of which belong to the structural class S8 of the serine protease family. Subtilisin E represents an unspecific protease of this structural class, while furin,

PC1 and PC5 are proteases with high substrate specificity. Compared to subtilisin several regions of insertions of three or more amino acids into the primary sequence of furin, PC1 and PC5 are seen. The regions marked with (-4-), (-5-), (-7-), (-9-) and (-11-) are preferred SDR insertion sites. These regions stretches fulfill two criteria to be selected as candidates for SDRs: firstly, they represent insertions in the specific proteases compared to the unspecific one and, secondly, they are close to the active site residues.

Figure 5: Three-dimensional structure of beta-secretase with the active site residues being shown in "ball-and-stick" representation and with the numbered regions indicating potential SDR insertion sites.

Figure 6: Alignment of the primary amino acid sequences of pepsin, b-secretase and cathepsin D, all of which belong to the structural class A1 of the aspartic protease family. Pepsin represents an unspecific protease of this structural class, while b-secretase and cathepsin D are proteases with high substrate specificity. Compared to pepsin several regions of insertions of three or more amino acids into the primary sequence of b-secretase and cathepsin D are seen. The regions marked with -1- to -11- correspond to possible SDR combining sites and are also marked in Fig.5.

Figure 7: illustrates the three-dimensional structure of caspase 7 with the active site residues being shown in "ball-and-stick" representation and with the numbered regions indicating potential SDR insertion sites.

Figure 8: shows the primary amino acid sequence of caspase 7 as a member of the cysteine protease class C14 family (see also SEQ ID NO: 14).

Figure 9: Schematic representation of method according to the third aspect of the invention.

Figure 10: Western blot analysis of trypsin expression. Supernatant of cell cultures expressing variants of trypsin are compared to negative controls. Lane 1: molecular weight standard; lane 2: negative control; lane 3: supernatant of variant a; lane 4: negative control; lane 5: supernatant of variant b. A primary antibody specific to the expressed protein and a secondary antibody for generation of the signal were used.

Figure 11: Time course of the proteolytic cleavage of a target substrate. Supernatant of cells containing the vector with the gene for human trypsin and that

of cells containing the vector without the gene was incubated with the peptide substrate described in the text. Cleavage of the peptide results in a decreased read out value. Proteolytic activity is confirmed for the positive clone.

Figure 12: Relative activity of three engineered proteolytic enzymes in comparison with human trypsin I on two different peptide substrates. A time course of the proteolytic digestion of the two substrates was performed and evaluated. Substrate B was used for screening and substrate A is a closely related sequence. Relative activity of the three variants was normalized to the activity of human trypsin I. Variant 1 and 2 clearly show increased specificity towards the target substrate. Variant 3, on the other hand, serves as a negative control with similar activities as the human trypsin I.

Figure 13: Relative specificities of trypsin and variants of engineered proteolytic enzymes with one or two SDRs, respectively. Activity of the proteases was determined in the presence and absence of competitor substrate, i.e. peptone at a concentration of 10mg/ml. Time courses for the proteolytic cleavage were recorded and the time constants k determined. The ratios between the time constants with and without competitor were formed and represent a quantitative measure for the specificity of the protease. The ratios were normalized to trypsin. The specificity of the variant containing two SDRs is 2.5 fold higher than that of the variant with SDR2 alone.

Figure 14: Shows the relative specificities of protease variants in absence and presence of competitor substrate. The protease variants containig two inserts with different sequences and the non-modified scaffold human trypsin I were expressed in a suitable host. Activity of the protease variants was determined as the cleavage rate of a peptide with the desired target sequence of TNF-alpha in the absence and presence of competitor substrate. Specificity is expressed as the ratio of cleavage rates in the presence and absence of competitor.

Figure 15: The figure shows the reduction of cytotoxicity induced by human TNF-alpha when incubating the human TNF-alpha with concentrated supernatant from cultures expressing the inventive engineered proteolytic enzymes being specific for human TNF-alpha. This indicates the efficacy of the inventive engineered proteolytic enzymes.

Figure 16: The figure shows the reduction of cytotoxicity induced by human TNF-alpha when incubating the human TNF-alpha with different concentrations of purified inventive engineered proteolytic enzyme being specific for human TNF-

alpha. Variant g comprises SEQ ID NO:72 as SDR1 and SEQ ID NO:73 as SDR2. This indicates the efficacy of the inventive engineered proteolytic enzymes.

Figure 17: The figure compares the activity of inventive engineered proteolytic enzymes being specific for human TNF-alpha with the activity of human trypsin I on two protein substrates: (a) human TNF-alpha; (b) mixture of human serum proteins. This indicates the safety of the inventive engineered proteolytic enzymes. Variant x corresponds to Seq ID No: 75 comprising the SDRs according to Seq ID No. 89 (SDR1) and 95 (SDR2). Variants xi and xii correspond to derivatives thereof comprising the same SDR sequences.

Figure 18: Specific hydrolysis of human VEGF by an engineered proteolytic enzyme derived from human trypsin.

Examples

In the following examples, materials and methods of the present invention are provided including the determination of catalytic properties of enzymes obtained by the method. It should be understood that these examples are for illustrative purpose only and are not to be construed as limiting this invention in any manner. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

In the experimental examples described below, standard techniques of recombinant DNA technology were used that were described in various publications, e.g. Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, or Ausubel et al. (1987), Current Protocols in Molecular Biology 1987-1988, Wiley Interscience. Unless otherwise indicated, restriction enzymes, polymerases and other enzymes as well as DNA purification kits were used according to the manufacturers specifications.

Example I: Identification of SDR sites in human trypsin

Insertion sites for SDRs have been identified in the serine protease human trypsin I (structural class S1) by comparison with members of the same structural class having a higher sequence specificity. Trypsin represents a member with low substrate specificity, as it requires only an arginine or lysine residue at the P_1 position. On the other hand, thrombin, tissue-type plasminogen activator or enterokinase all have a high specificity towards their substrate sequences, i.e. (L/I/V/F)XPR^ NA, CPGR^ VVGG and DDDK^ , respectively. The primary sequences and tertiary structures of these and further S1 serine proteases have been aligned

in order to determine regions of low and high sequence and structure homology and especially regions that correspond to insertions in the sequences of the more specific proteases (Figure 2). Several regions of insertions equal or longer than 3 amino acids representing potential SDR sites have been identified as indicated in Figure 1. These regions were chosen as target sites for the insertion of SDRs in the examples below, e.g. SDR1 (region one in figure 2, after amino acid 42 according to SEQ ID NO:1) with a length of six and SDR2 (region three in figure 2, after amino acid 123 according to SEQ ID NO:1) with a length of five amino acids, respectively.

Example II: Molecular cloning of the human trypsin I gene to be used as scaffold protein and expression of the mature protease in B. subtilis

The gene encoding the unspecific protease human trypsinogen I was cloned into the vector pUC18. Cloning was done as follows: the coding sequence of the protein was amplified by PCR using primers that introduced a KpnI site at the 5' end and a BamHI site at the 3' end. This PCR fragment was cloned into the appropriate sites of the vector pUC18. Identity was confirmed by sequencing. After sequencing the coding sequence of the mature protein was amplified by PCR using primers that introduced different BglI sites at the 5' end and the 3' end.

This PCR fragment was cloned into the appropriate sites of an E. coli – B. subtilis shuttle vector. The vector contains a pMB1 origin for amplification in E. coli, a neomycin resistance marker for selection in E. coli, as well as a P43 promoter for the constitutive expression in B. subtilis. A 87 bp fragment that contains the leader sequence encoding the signal peptide from the sacB gene of B. subtilis was introduced behind the P43 promoter. Different BglI restriction sites serve as insertion sites for heterologous genes to be expressed.

Expression of human trypsin I was confirmed by measurement of the proteolytic aciticity in supernatant of cells containing the vector with the gene in comparison to a negative control. A peptide including an arginine cleavage site was chosen as a substrate. The peptide was N-terminally biotinylated and labeled with a fluorophore at the C-terminus. After incubation of the peptide with culture supernatant streptavidin was added. Uncleaved peptide associate with streptavidin and lead to a high read out value while cleavage results in low read out values. Figure 11 shows the time course of a proteolytic digestion of B. subtilis cells containing the vector with the trypsin I gene in comparison to B. subtilis cells containing the vector without the trypsin I gene (negative control).

As a further confirmation of expression of the protease, supernatants of cells containing the vector with the gene and control cells were analyzed by polyacrylamid gel electrophoreses and subsequent western blot using an antibody specific to the target protease. The procedure was performed according to standard methods (Sambrook, J.F; Fritsch, E.F.; Maniatis,T.; Cold Spring Harbor Laboratory Press, Second Edition, 1989, New York). Figure 8 confirms expression of the protein only in the cells harbouring the vector with the gene for trypsin.

Example III: Providing a scaffold protein

In this example, human trypsin I was used as the scaffold protein. The gene was either used in its natural form, or, alternatively, was modified to result in a scaffold protein with increased catalytic activity or further improved characteristics.

The modification was done by random modification of the gene, followed by expression of the enzyme and subsequent selection for increased activity. First, the gene was PCR amplified under error-prone conditions, essentially as described by Cadwell, R.C and Joyce, G.F. (PCR Methods Appl. 2 (1992) 28-33). Error-prone PCR was done using 30 pmol of each primer, 20 nmol dGTP and dATP, 100 nmol dCTP and dTTP, 20 fmol template, and 5 U Taq DNA polymerase in 10 mM Tris HCl pH 7.6, 50 mM KCl, 7 mM MgCl2, 0.5 mM MnCl2, 0.01 % gelatin for 20 cycles of 1 min at 94 °C, 1 min at 65 °C and 1 min at 72 °C. The resulting DNA library was purified using the Qiaquick PCR Purification Kit following the suppliers' instructions. The PCR product was digested with the restriction enzyme *BglI* and purified. Afterwards, the PCR product was ligated into the E. coli – B. subtilis shuttle vector described above which was digested with BglI and dephosphorylated. The ligation products were transformed into E. coli, amplified in LB, and the plasmids were purified using the Qiagen Plasmid Purification Kit following the suppliers' instructions. Resulting plasmids were transformed into B. subtilis cells.

Alternatively, or in addition to random mutagenesis, variants of the gene were statistically recombined at homologous positions by use of the Recombination Chain Reaction, essentially as described in WO 0134835. PCR products of the genes encoding the protease variants were purified using the QIAquick PCR Purification Kit following the suppliers' instructions, checked for correct size by agarose gel electrophoresis and mixed together in equimolar amounts. 80 µg of this PCR mix in 150 mM TrisHCl pH 7.6, 6.6 mM $MgCl_2$ were heated for 5 min at 94 °C and subsequently cooled down to 37 °C at 0.05 °C/s in order to re-anneal strands and thereby produce heteroduplices in a stochastic manner. Then, 2.5 U Exonuclease III

per µg DNA were added and incubated for 20, 40 or 60 min at 37 °C in order to digest different lengths from both 3' ends of the heteroduplices. The partly digested PCR products were refilled with 0.6 U Pfu polymerase per µg DNA by incubating for 15 min at 72 °C in 0.17 mM dNTPs and Pfu polymerase buffer according to the suppliers' instructions. After performing a single PCR cycle, the resulting DNA was purified using the QIAquick PCR Purification Kit following the suppliers' instructions, digested with BglI and ligated into the linearized vector. The ligation products were transformed into E. coli, amplified in LB containing ampicillin as marker, and the plasmids were purified using the Qiagen Plasmid Purification Kit following the suppliers' instructions. Resulting plasmids were transformed into B. subtilis cells.

Example IV: Insertion of SDRs into the protein scaffold of human trypsin I and generation of an engineered proteolytic enzyme with specificity for a peptide substrate having the sequence KKWLGRVPGGPV.

In order to create insertion sites for SDRs in human trypsin I, two pairs of different restriction sites were introduced into the gene at sites that were identified as potential SDR sites (see Example I above) without changing the amino acid sequence. The insertion of the restriction sites was done by overlap extension PCR. Primers restr1 and restr2 were used for the introduction of SacII and BamHI restriction sites, restr3 and restr4 were used for the introduction of KpnI and NheI restriction sites. The sequences of the primers were as follows:

Binding site for restr1 and restr2 and the corresponding amino acid sequence (SEQ ID NO:54):

5'-GGTGGTATCAGCAGGCCACTGCTACAAGTCCCGCATCCAGGT-3'

V V S A G H C Y K S R I Q

Forward primer restr1 (SEQ ID NO:56):

5'-GGTGGTATCCGCGGGCCACTGCTACAAGTCCCGGATCCAGGT-3'

Reverse primer restr2 (SEQ ID NO:57):

5'-ACCTGGATCCGGGACTTGTAGCAGTGGCCCGCGGATACCACC-3'

Binding site for restr3 and restr4 and the corresponding amino acid sequence (SEQ ID NO:58):

5'-CCACTGGCACGAAGTGCCTCATCTCTGGCTGGGGCAACACTGCGAGCTCT-3'

T G T K C L I S G W G N T A S S

Forward primer restr3 (SEQ ID NO:60):

5'-CCACTGGCACGAAGTGCCTCATCTCTGGCTGGGGCAACACTGCGAGCTCT-3'

Reverse primer restr4 (SEQ ID NO:61):

5'-AGAGCTAGCAGTGTTGCCCCAGCCAGAGATGAGGCACTTGGTACCAGTGG-3'

In a first overlap extension PCR, the SacII/BamHI sites were introduced, enabling to insert SDR1, and in a second overlap extension PCR the KpnI/NheI sites, enabling the insertion of SDR2. The product of the overlap extension PCR was amplified using primers pUC-forward and pUC-reverse. The sequences of pUC-forward and pUC-reverse are as follows:

pUC-forward (SEQ ID NO:62): 5'-GGGGTACCCCACCACCATGAATCCACTCCT-3'

pUC-reverse (SEQ ID NO:63): 5'-CGGGATCCGGTATAGAGACTGAAGAGATAC-3'

The restriction sites generated thereby were subsequently used to insert defined or random oligonucleotides into the SDR1 and SDR2 insertion sites by standard restriction and ligation methods. Typically, two complementary synthetic 5'-phosphorylated oligonucleotides were annealed and ligated into a vector carrying the modified human trypsin I gene that was cleaved with the respective restriction enzymes. Oligonucleotides encoding SDR1 were inserted via the SacII/BamHI sites whereas oligonucleotides encoding SDR2 were inserted via the KpnI/NheI sites. For each insertion an oligonucleotide pair according to the following general sequences was used ([P] indicating 5'-phosphorylation, N and X indicating any nucleotide or amino acid residue, respectively):

oligox-SDR1f (SEQ ID NO:64):

5'-[P]-GGGCCACTGCTACNNNNNNNNNNNNNNNNNNAAGTCCCG-3'

oligox-SDR1r (SEQ ID NO:66):

3'-CGCCCGGTGACGATGNNNNNNNNNNNNNNNNNNTTCAGGGCCTAG-[P]-5'

G H C Y X X X X X X K S

oligox-SDR2f (SEQ ID NO:67):

5'-[P]-CAAGTGCCTCATCTCTGGCTGGGGCAACNNNNNNNNNNNNNNNNACTG-3'

oligox-SDR2r (SEQ ID NO:69):

3'-CATGGTTCACGGAGTAGAGACCGACCCCGTTGNNNNNNNNNNNNNNNNTGACGATC-[P]-5'

K C L I S G W G N X X X X X T

As an alternative to the above method, a PCR based method was used for the integration of random-sequences into the SDR1 and SDR2 insertion sites in the modified human trypsin I. For each SDR, one primer was used where the SDR region is fully randomized. Sequences of the primers were as follows (N = A/C/G/T, B = C/G/T, V = A/C/G):

Primer SDR1-mutnnb-forward (SEQ ID NO:70):

5'-TGGTATCCGCGGGCCACTGCTACNNBNNBNNBNNBNNBNNBAAGTCCCGGATCCAGGTG-3'

Primer SDR2-mutnnb-reverse (SEQ ID NO:71):

5'-GGCGCCAGAGCTAGCAGTVNNVNNVNNVNNVNNGTTGCCCCAGCCAGAGATG-3'

The codon NNB, or VNN in the reverse strand, allows all 20 amino acids to made, but reduces the probability of encoding a stop codon from 0.047 to 0.021.

As a further alternative, after identification of SDRs that lead to increased specificity, these SDRs were used as templates for further randomization. Thereby, random peptide sequences were inserted that were partially randomized at each position and partially identical at each position to the original sequence.

As an example, random peptide sequences that have in approximately 1 of 3 cases the template amino acid residue and in approximately 2 of 3 cases any other amino acid residue at each position were inserted into the two SDR insertion sites of the modified human trypsin I. For this purpose, primers that contain at each nucleotide position of the SDR approximately 70% of the template bases and 30% of a mixture of the three other bases were used.

With each primer pair a PCR was performed under standard conditions using the human trypsin I gene as template. The resulting DNA was purified using the QIAquick PCR Purification Kit following the suppliers' instructions and digested with SacII and NheI. After digestion the DNA was purified and ligated into the SacII and NheI digested and dephosphorylayted vector. The ligation products were transformed into E. coli, amplified in LB containing the respective marker, and the plasmids were purified using the Qiagen Plasmid Purification Kit following the suppliers' instructions. Resulting plasmids were transformed into B. subtilis cells. These cells were then separated to single cells, grown to clones, and after expression of the protease gene screened for proteolytic activity.

The following substrates were employed for screening for proteolytic activity (SEQ ID NOs:76 and 77):

substrate A	L	L	W	L	G	R	V	V	G	G	P	V
substrate B	K	K	W	L	G	R	V	P	G	G	P	V

Protease variants were screened on substrate B at complexities of 10^6 variants by confocal fluorescence spectroscopy. The substrate was a peptide biotinylated at the N-terminus and fluorescently labeled at the C-terminus. After incubation of the peptide with supernatant of cells expressing different variants of the protease, streptavidin is added and the samples are analysed by confocal fluorimetry. The low concentration of the peptide (20nM) leads to a preferential cleavage by proteases with a high k_{cat}/K_M value, i.e. proteases with high specificity towards the target sequence.

Variants selected in the screening procedure were further evaluated for their specificity towards substrate B and closely related substrate A by measuring time courses of the proteolytic digestion and determining the rate constants which are proportional to the k_{cat}/K_M values. Clearly, compared to the human trypsin that was used as scaffold protein, the specific activity of variants 1 and 2 is shifted (SEQ ID NOs: 2 and 3, respectively) towards substrate B. Variant 3 (SEQ ID NO:4), on the other hand, serves as a negative control with similar activities as the human trypsin I. Sequencing of the genes of the three variants revealed the following amino acid sequences in the SDRs.

Table 2: Sequences of the two SDRs in three different variants selected for specific hydrolysis of substrate B (SEQ ID NOs:78-83).

	SDR 1						SDR 2				
Trypsin	-	-	-	-	-	-	-	-	-	-	-
Variant 1	D	A	V	G	R	D	T	I	T	N	S
Variant 2	N	G	R	D	L	E	V	R	G	T	W
Variant 3	G	F	V	M	F	N	R	S	P	L	T

In a further experiment a pool of variants containing different numbers of SDRs per gene were screened for increased specificity using a mixture of the defined substrate and pepton as a competing substrate. Variants containing one or two SDRs per gene have been analyzed further. As a measure for the specificity the activity in the peptide cleavage assay was compared with and without the presence of the competing substrate. The concentration of the competing substrate was 10mg/ml. Under these conditions, unspecific proteases show, compared to specific proteases, a stronger decrease in activity with increasing competitor concentrations (range between 0 and 100mg/ml). The ratio of proteolytic activity with and without substrate is a quantitative measure for the specificity of the proteases. Figure 9 shows the relative activities with and without competing substrate. Human trypsin I that was used as the scaffold protein and two variants, one containing only SDR2, and one containing both SDRs, were compared. The specificity of the variant with both SDRs is by a factor of 2.5 higher than that of the variant with SDR2 only, confirming that there is a direct relation between the number of SDRs and the quantitative specificity of resulting engineered proteolytic enzymes.

Example V: Generation of an engineered proteolytic enzyme that specifically inactivates human TNF-alpha

Human trypsin alpha I or a derivative comprising one or more of the following amino acid substitutions E56G; R78W; Y131F; A146T; C183R was used as protein scaffold for the generation of an engineered proteolytic enzyme with high specificity towards human TNF-alpha. The identification of SDR sites in human trypsin I or derivatives thereof was done as described above. Two insertion sites within the scaffold were choosen for SDRs. The protease variants containing two inserts with different sequences and also the human trypsin I itself with no inserts were expressed in a *Bacillus subtilis* cells. The variant protease cells were separated to single cell clones and the protease expressing variants were screened for proteolytic activity on peptides with the desired target sequence of TNF-alpha. The activity of the protease variants was determined as the cleavage rate of a peptide with the desired target sequence of TNF-alpha in the absence and presence of competitor substrate. The specificity is expressed as the ratio of cleavage rates in the presence and absence of competitor (Fig. 14).

Table 3: Relative specificity of variants of engineered proteolytic enzymes with different SDR sequences in absence and presence of competitor substrate (SEQ ID NOs:84-95).

	k with comp./ k without comp	Seq. of SDR 1	Seq. of SDR 2
scaffold (no SDRs)	0.092	---	----
variant a	0.130	RPWDPS	VHPTS
variant b	0.187	GFVMFN	RSPLT
variant c	0.235	EIANRE	RGART
variant d	0.310	KAVVGT	RTPIS
variant e	0.374	VNIMAA	TTARK
variant f	0.487	AAFNGD	RKDFW

The antagonistic effect of three inventive protease variants on human TNF-alpha is shown in Figure 15. By the use of the variants, the induction of apoptosis is almost completely eliminated indicating the anti-inflammatory efficacy of the inventive proteases to initiate TNF-alpha break down. TNF-alpha has been incubated with concentrated supernatant from cultures expressing the variants i to iii for 2 hours. The resulting TNF-alpha has been incubated with non-modified cells for 4 hours. The effect of the remaining TNF-alpha activity was determined as the extent of apoptosis induction by detection of activated caspase-3 as marker for apoptotic cells. For the controls either no protease was added with the human TNF-alpha (dead cells) or buffer instead of human TNF-alpha (live cells) was used, respectively. An analogous experiment is shown in Figure 16 using purified variant

xiii. TNF-alpha was incubated with different concentrations of the purified inventive protease variant.

To demonstrate the specificity of the inventive protease variants, proteins from human blood serum or purified human TNF-alpha have been incubated with human trypsin I or the inventive engineered proteolytic enzyme variants, respectively. Here, variant x corresponds to Seq ID No: 75 comprising the same SDRs as variant f, i.e. SDRs according to Seq ID No. 89 (SDR1) and 95 (SDR2). Variants xi and xii correspond to derivatives thereof comprising the same SDR sequences. Remaining intact protein was was determined as a function of time. While the variants as well as human trypsin I digest human TNF-alpha, only trypsin shows activity on serum protein (Figure 17 a and b). This demonstrates the high TNF-alpha specificity of the inventive proteolytic enzymes and indicates their safety and accordingly their low side effects for therapeutic use.

Example VI: Generation of an engineered proteolytic enzyme that specifically hydrolysis human VEGF.

Human trypsin I was used as protein scaffold for the generation of an engineered proteolytic enzyme with high specificity towards human VEGF. The identification of SDR sites in human trypsin I was done as described above. Two insertion sites within the scaffold were choosen for SDRs. The protease variants containing two inserts with different sequences were expressed in *Bacillus subtilis* cells. The variant protease cells were separated to single cell clones and the protease expressing variants were screened as described above. The activity of the protease variants was determined as the rate of VEGF cleavage. 4µg of recombinant human VEGF165 was incubated with 0.18 µg of purified protease in PBS / pH 7.4 at room temperature. Aliquots were taken at the indicated time points and analysed on a polyacrylamide gel. The extend of cleavage was quantified by densitometric analysis of the bands. The activity is plotted over incubation time in Figure 18. Specific cleavage was controlled by further SDS polyacrylamide gel analyses.

Claims

1. Use of a protease with defined specificity for a target substrate for preparing a medicament for the treatment of a specific disease related to said target substrate.

2. The use of claim 1, wherein the protease is an engineered protease,preferably an engineered protease characterized by a combination of the following components:
(a) a protein scaffold capable to catalyze at least one chemical reaction on at least one target substrate and being derived from one or more proteins, and
(b) one or more specificity determining regions (SDRs) located at sites in the protein scaffold that enable the resulting engineered protein to discriminate between at least one target substrate and one or more different substrates, and wherein the SDRs are essentially synthetic peptide sequences.

3. The use of claim 2, wherein
(I) the SDRs (b) have a length between one and 50 amino acid residues, preferably have a length between 2 and 20 amino acid residues, more preferably a length between 2 and 10 amino acid residues, even more preferably a length between 3 and 8 amino acid residues, and wherein the number of SDRs is at least one, preferably more than one, more preferably between two and eleven, most preferably between two and six; and/or
(II) the protein scaffold (a) is comprised of one or more polypeptide segments being derived from same or different
(i) proteins encoded by a gene of viral or prokaryotic or eukaryotic origin, and/or
(ii) native enzymes, mutated variants or truncated derivates thereof, and/or
(iii) mammalian enzymes, preferably human enzymes.

4. The use of claim 2 or 3, wherein the protein scaffold (a) is derived from a protease selected from the group consisting of aspartic, cysteine, serine, metallo and threonine proteases,
even more preferably the protein scaffold (a) is derived from a serine protease of the structural class S1, S8, S11, S21, S26, S33 or S51, most preferably from class S1 or S8, a cysteine protease of the structure class C1, C2, C4, C10, C14, C19, C47, C48 or C56, most preferably from class C14, or an aspartic protease of the structural class A1, A2 or A26, most preferably from class A1, or a metalloprotease of the strucutral class M4 or M10.

5. The use according to any one of claims 2 to 4, wherein

(i) the protein scaffold (a) is derived from a serine protease of the structural class S1; and/or

(ii) the SDRs are located at one or more positions selected from the group of positions that correspond structurally or by amino acid sequence homology to the regions 18-25, 38-48, 54-63, 73-86, 122-130, 148-156, 165-171 and 194-204 in human trypsin I having the amino acid sequence shown in SEQ ID NO:1, and preferably at one or more positions selected from the group of positions that correspond structurally or by amino acid sequence homology to the regions 20-23, 41-45, 57-60, 76-83, 125-128, 150-153, 167-169 and 197-201 in human trypsin I.

6. The use of claim 5, wherein

(i) the protein scaffold (a) is derived from the serine protease trypsin, preferably human trypsin I having the amino acid sequence shown in SEQ ID NO:1, or the amino acid sequence SEQ ID NO:1 comprising one or more of the following amino acid substitutions E56G, R78W, Y131F, A146T and C183R; and

(ii) at least one of two SDRs are located in the scaffold, a first SDR having a length of up to 6 amino acids and being inserted between residues 42 and 43, and a second SDR having a length of up to 5 amino acids and bein inserted between residues 123 and 124 (numbering relative to human trypsin having the amino acid sequence shown in SEQ ID NO:1).

7. The use of claim 6, wherein

(i) one of the peptide sequences of the following group: SEQ ID NO: 72, 78, 79, 80, 84, 85, 86, 87, 88, and 89 is inserted as the first SDR between residues 42 and 43; and/or one of the peptide sequences of the following group: SEQ ID NO: 73, 81, 82, 83, 90, 91, 92, 93, 94, and 95 is inserted as the second SDR between residues 123 and 124; or

(ii) the engineered enzyme comprises an amino acid sequence as shown in SEQ ID NO: 74, or SEQ ID NO: 75.

8. The use according to any one of claims 2 to 7, wherein

(i) the protein scaffold (a) is derived from a serine protease of the structural class S8; and/or

(ii) the SDRs are located at one or more positions selected from the group of positions that correspond structurally or by amino acid sequence homology to the regions 6-17, 25-29, 47-55, 59-69, 101-111, 117-125, 129-137, 139-154, 158-169, 185-195 and 204-225 in subtilisin E from *Bacillus subtilis* having the amino

acid shown in SEQ ID NO:7, and preferably at one or more positions selected from the group of positions that correspond structurally or by amino acid sequence homology to the regions 59-69, 101-111, 129-137, 158-169 and 204-225in subtilisin E for *Bacillus subtilis*.

9. The use according to any one of claims 2 to 7, wherein
(i) the protein scaffold (a) is derived from an aspartic protease of the structural class A1; and/or
(ii) the SDRs are located at one or more positions selected from the group of positions that correspond structurally or by amino acid sequence homology to the regions 6-18, 49-55, 74-83, 91-97, 112-120, 126-137, 159-164, 184-194, 242-247, 262-267 and 277-300 in human pepsin having the amino acid sequence shown in SEQ ID NO:11, and more preferably at one or more positions selected from the group of positions that correspond structurally or by amino acid sequence homology to the regions 10-15, 75-80, 114-118, 130-134, 186-191 and 280-296 in human pepsin.

10. The use according to any one of claims 2 to 7, wherein
(i) the protein scaffold (a) is derived from a cysteine protease of the structural class C14; and/or
(ii) the SDRs are located at one or more positions selected from the group of positions that correspond structurally or by amino acid sequence homology to the regions 78-91, 144-160, 186-198, 226-243 and 271-291 in human caspase 7 having the amino acid sequence of SEQ ID NO:14, and preferably at one or more positions selected from the group of positions that correspond structurally or by amino acid sequence homology to the regions 80-86, 149-157, 190-194 and 233-238 of human caspase 7.

11. The use according to any one of claims 2 to 11, wherein the protease is obtainable by a method comprising at least the following steps:
(a) providing a protein scaffold which catalyzes at least one chemical reaction on at least one target substrate,
(b) generating a library of enzymes or isolated enzymes by combining the protein scaffold from step (a) with variants of one or more fully or partially random peptide sequences at sites in the protein scaffold that enable the resulting enzyme to discriminate between at least one target substrate and one or more different substrates, and

(c) selecting out of the (library of) enzymes generated in step (b) one or more enzymes that have defined specificities towards at least one target substrate.

12. The use according to any one of claims 1 to 11, wherein
(I) the protease hydrolyzes the target substrate and thereby
(i) eliminates or reduces one or more biological activities or physico-chemical properties or pharmacological properties of the target protein, and/or
(ii) activates or increases one or more biological activities or physico-chemical properties or pharmacological properties of the target protein, and/or
(iii) adds one or more biological activities or physico-chemical properties or pharmacolocical properties to the target protein;
and/or
(II) the target substrate hydrolyzed by the protease is a soluble protein, in particular a cytokine, such as the TNF-superfamily proteins, interleukines, interferons, chemokines and growth factors; a hormone; a toxin; an enzyme, such as oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases; a structural protein, such as collagens; an immunoglobulin; an activity modulating protein; a DNA binding protein; or a membrane associated protein, in particular a single pass transmembrane protein; a multipass transmembrane protein, such as G-protein coupled receptors, ion channels and transporters; a lipid-anchored membrane protein or a GPI-anchored membrane protein.

13. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is a type-1 single pass transmembrane protein, preferably
(i) the protease is capable of hydrolyzing peptide bonds in human Vascular endothelial growth factor receptor 2 (hVEGFR-2) or related molecules of the same structural class, preferably the peptide bonds between positions 13/14, 16/17, 19/20, 23/24, 33/34, 39/40, 53/54, 61/62, 88/89, 110/111, 112/113, 113/114, 115/116, 119/120, 120/121, 179/180, 184/185, 198/199, 203/204, 204/205, 208/209, 220/221, 245/246, 256/257, 260/261, 261/262, 291/292, 293/294, 294/295, 295/296, 298/299, 299/300, 301/302, 302/303, 307/308, 310/311, 311/312, 317/318, 322/323, 327/328, 336/337 and/or 339/340, most preferred between positions 39/40, 53/54, 61/62, 88/89, 119/120, 120/121, 204/205, 260/261, 293/294, 298/299, 299/300, 302/303 and/or 336/337 in hVEGFR-2 (SEQ ID NO: 186) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of cancer or other diseases connected with hVEGFR-2.

14. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is a TNF-superfamily protein, preferably
(i) the protease is capable of hydrolyzing peptide bonds in human B lymphocyte stimulator (hBlys) or related molecules of the same structural class, preferably the peptide bonds between positions 160/161, 163/164, 173/174, 174/175, 181/182, 188/189, 192/193, 204/205, 206/207, 214/215, 215/216, 216/217, 222/223, 231/232, 252/253, 257/258, 265/266 and/or 273/274 most preferred between positions 160/161, 163/164, 181/182, 188/189 and/or 222/223 in hBlys (SEQ ID NO: 192) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of acute or chronic inflammatory diseases like rheumatoid arthritis, inflammatory bowel diseases, psoriasis, Crohn's disease, Ulcerative colitis, Sjogren's syndrome, systemic lupus erythematosus, multiple sclerosis, Systemic inflammatory response syndrome (SIRS), Graft-versus-host disease (GvhD) or sclerodermia, or for treatment of B-cell malignancies like classical Hodgkin's Lymphoma (cHL), mantle cell lymphoma, centrocytic lymphoma (CCL) or follicular lymphoma, or other diseases connected with hBlys.

15. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is an interleukine, preferably
(i) the protease is capable of hydrolyzing peptide bonds in human Oncostatin M (hOSM) or related molecules of the same structural class, preferably the peptide bonds between positions 11/12, 19/20, 22/23, 26/27, 32/33, 36/37, 41/42, 44/45, 46/47, 47/48, 50/51, 52/53, 59/60, 60/61, 67/68, 68/69, 84/85, 97/98, 99/100, 100/101, 106/107, 107/108, 109/110, 122/123, 126/127, 133/134, 158/159, 162/163, 163/164 and/or 175/176, most preferred between positions 19/20, 44/45, 47/48, 60/61, 67/68, 97/98, 100/101, 109/110, 126/127, 133/134, 162/163 and/or 175/176 in hOSM (SEQ ID NO: 178) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of cancer like prostate cancer or other diseases connected with hOSM.

16. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is a hydrolase, preferably
(i) the protease is capable of hydrolyzing peptide bonds in human matrix metalloproteinase-7 (hMMP-7) or related molecules of the same structural class,

preferably the peptide bonds between positions 13/14, 22/23, 24/25, 25/26, 33/34, 37/38, 44/45, 45/46, 51/52, 52/53, 55/56, 66/67, 73/74, 76/77, 100/101, 101/102, 102/103, 103/104, 106/107, 133/134, 146/147, 151/152, 155/156, 162/163 and/or 166/167, most preferred between positions 24/25, 33/34, 51/52, 55/56, 73/74, 76/77, 101/102, 133/134 and/or 146/147in hMMP-7 (SEQ ID NO: 184) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of cancer or other diseases connected with hMMP-7.

17. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is a growth factor.

18. The use according to claim 17, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human epidermal growth factor (hEGF) or related molecules of the same structural class, preferably the peptide bonds between positions 11/12, 13/14, 17/18, 22/23, 27/28, 28/29, 40/41, 41/42, 44/45, 45/46, 46/47, 49/50 and/or 50/51, most preferred between positions 11/12, 17/18, 44/45 an/or 49/50 in hEGF (SEQ ID NO: 156) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of carcinomas, solid cancers like breast, colon or stomach cancer, or other diseases connected with hEGF.

19. The use according to claim 17, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human fibroblast growth factor (hFGF-1) or related molecules of the same structural class, preferably the peptide bonds between positions 28/29, 35/36, 36/37, 37/38, 39/40, 49/50, 60/61, 70/71, 74/75, 81/82, 94/95, 100/101, 101/102, 104/105, 105/106, 112/113, 113/114, 119/120, 122/123, 125/126 and/or 128/129, most preferred between positions 28/29, 35/36, 70/71, 81/82, 100/101, 104/105, 113/114 adnd/or 122/123 in hFGF-1 (SEQ ID NO: 157) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of cancer, angiogenesis or other diseases connected with hFGF-1.

20. The use according to claim 17, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human substance P (substance P) or related molecules of the same structural class, preferably the

peptide bonds between positions 1/2, 3/4, 7/8 and/or 8/9 in substance P(SEQ ID NO: 164) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of cancers like small cell lung cancer, colorectal cancer, astrocytic/glial brain tumors or other diseases connected with substance P.

21. The use according to claim 17, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human Bradykinin (Bradykinin) or related molecules of the same structural class, preferably the peptide bonds between positions 1/2, 5/6 and/or 8/9 in Bradykinin (SEQ ID NO: 165) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of vascular and neuro-glial pathology in diabetic retinopathy, cerebral ischemia and trauma, hyperalgesia, inflammatory diseases or conditions, asthma and cancer, pain, pathological vascular leakage or vasodilation, pathological contraction of various smooth muscles, pathological cell proliferation or other diseases connected with Bradykinin.

22. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is a transferase.

23. The use according to claim 22, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human tyrosine protein kinase (hSrc) or related molecules of the same structural class, preferably the peptide bonds between positions 15/16, 22/23, 25/26, 59/60, 65/66, 87/88, 94/95, 99/100, 102/103, 123/124, 124/125, 126/127, 135/136, 147/148, 150/151, 153/154, 158/159, 176/177, 178/179, 182/183, 200/201, 216/217, 223/224, 234/235, 249/250, 261/262, 266/267, 271/272, 275/276, 277/278, 297/298, 327/328, 331/332, 333/334, 337/338, 354/355, 356/357, 378/379, 387/388, 397/398, 391/392, 395/396, 398/399, 407/408, 411/412, 418/419, 419/420, 420/421, 422/423, 423/424 and/or 436/437, most preferred between positions 59/60, 123/124, 126/127, 135/136, 176/177, 182/183, 200/201, 275/276, 277/278, 331/332, 354/355, 387/388, 391/392, 395/396, 418/419 and/or 423/424 in hSrc (SEQ ID NO: 162) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of cancer, osteoporosis or other diseases connected with hSrc.

24. The use according to claim 22, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human RAC-beta serine/threonine protein kinase (hAkt-2) or related molecules of the same structural class, preferably the peptide bonds between positions 9/10, 15/16, 25/26, 26/27, 27/28, 39/40, 63/64, 71/72, 72/73, 78/79, 79/80, 98/99, 99/100, 100/101, 104/105, 105/106, 106/107, 120/121, 124/125, 125/126, 141/142, 170/171, 178/179, 179/180, 181/182, 182/183, 184/185, 206/207, 209/210, 211/212, 212/213, 220/221, 221/222, 223/224, 226/227, 242/243, 243/244, 245/246, 256/257, 260/261, 262/263, 275/276, 276/277, 282/283, and /or 292/293, most preferred between positions 27/28, 39/40, 41/42, 72/73, 78/79, 100/101, 105/106, 106/107, 125/126, 179/180, 184/185, 209/210, 223/224, 245/246, 262/263 and/or 282/283 in hAkt-2 (SEQ ID NO: 163) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of cancer or other diseases connected with hAkt-2.

25. The use according to claim 22, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human glycogen synthase kinase-3-beta (hGSK-3) or related molecules of the same structural class, preferably the peptide bonds between positions 16/17, 19/20, 24/25, 26/27, 43/44, 52/53, 57/58, 60/61, 62/63, 68/69, 69/70, 87/88, 88/89, 89/90, 90/91, 91/92, 107/108, 110/111, 112/113, 114/115, 116/117, 156/157, 158/159, 175/176, 177/178, 182/183, 186/187, 187/188, 189/190, 226/227, 230/231, 234/235, 244/245, 245/246, 248/249, 249/250, 254/255, 256/257, 263/264, 269/270, 272/273, 274/275, 307/308, 308/309, 311/312, 315/316 and/or 321/322, most preferred between positions 57/58, 87/88, 88/89, 89/90, 90/91, 114/115, 116/117, 158/159, 175/176, 182/183, 230/231, 244/245, 248/249, 254/255, 256/257, 274/275 and/or 321/322 in hGSK-3 (SEQ ID NO: 167) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of diabetes or other diseases connected with hGSK-3.

26. The use according to claim 22, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human cyclin-dependent protein kinase-2 (hcdk-2) or related molecules of the same structural class, preferably the peptide bonds between positions 8/9, 9/10, 12/13, 15/16, 19/20, 22/23, 24/25, 34/35, 50/51, 57/58, 68/69, 73/74, 75/76, 88/89, 89/90, 92/93,

122/123, 138/139, 162/163, 178/179, 179/180, 180/181, 199/200, 200/201, 206/207, 208/209, 210/211, 217/218, 223/224, 224/225, 237/238, 242/243, 245/246, 247/248, 250/251, 273/274 and/or 291/292, most preferred between positions 12/13, 50/51, 57/58, 73/74, 138/139, 180/181, 200/201, 206/207, 223/224, 242/243, 247/248 and/or 273/274 in hcdk-2 (SEQ ID NO: 168) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of cancer or other diseases connected with hcdk-2.

27. The use according to claim 22, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human Mitogen-activated protein kinase p38-alpha (hp38-kinase) or related molecules of the same structural class, preferably the peptide bonds between positions 8/9, 11/12, 14/15, 21/22, 48/49, 53/54, 56/57, 66/67, 93/94, 96/97, 97/98, 117/118, 120/121, 123/124, 124/125, 159/160, 160/161, 162/163, 172/173, 175/176, 176/177, 177/178, 181/182, 199/200, 219/220, 229/230, 232/233, 236/237, 244/245, 247/248, 248/249, 252/253, 255/256, 257/258, 285/286, 286/287, 293/294, 294/295, 310/311, 312/313, 314/315, 315/316, 316/317, 320/321, 323/324, 329/330, 330/331, 334/335, 335/336, 341/342, 342/343 and/or 343/344, most preferred between positions 48/49, 56/57, 93/94, 96/97, 123/124, 160/161, 175/176, 236/237, 247/248, 255/256, 257/258, 294/295, 314/315, 315/316, 329/330, 330/331, 334/335 and/or 342/343 in hp38-kinase (SEQ ID NO: 187) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of intimal hyperplasia, vascular remodeling upon blood vessel injury or other diseases connected with hp38-kinase.

28. The use according to claim 22, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human Stress-activated protein kinase JNK3 (hJNK3-kinase) or related molecules of the same structural class, preferably the peptide bonds between positions 11/12, 19/20, 38/39, 43/44, 44/45, 53/54, 65/66, 72/73, 90/91, 93/94, 94/95, 106/107, 116/117, 118/119, 120/121, 124/125, 134/135, 179/180, 196/197, 197/198, 198/199, 214/215, 216/217, 222/223, 223/224, 233/234, 244/245, 245/246, 251/252, 253/254, 255/256, 259/260, 267/268, 271/272, 277/278, 279/280, 282/283, 302/303, 307/308, 308/309, 318/319, 319/320, 325/326, 338/339, 339/340, 344/345 and/or 345/346, most preferred between positions 38/39, 90/91, 93/94, 116/117,

179/180, 216/217, 222/223, 255/256, 259/260, 267/268, 271/272, 277/278, 279/280, 318/319, 319/320 and/or 339/340 in hJNK3-kinase (SEQ ID NO: 188) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of intimal hyperplasia, vascular remodeling upon blood vessel injury or other diseases connected with hJNK3-kinase.

29. The use according to claim 22, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human poly (ADP-ribose) polymerase-1 (hPARP) or related molecules of the same structural class, preferably the peptide bonds between positions 13/14, 19/20, 22/23, 23/24, 27/28, 29/30, 34/35, 39/40, 42/43, 43/44, 65/66, 70/71, 74/75, 82/83, 86/87, 87/88, 114/115, 118/119, 122/123, 126/127, 133/134, 134/135, 141/142, 144/145, 145/146, 146/147, 148/149, 149/150, 158/159, 179/180, 181/182, 188/189, 196/197, 222/223, 270/271, 272/273, 282/283, 304/305, 307/308 and/or 320/321, most preferred between positions 22/23, 43/44, 118/119, 122/123, 145/146, 146/147, 148/149, 179/180 and/or 272/273 in hPARP (SEQ ID NO: 175) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic cell death, reperfusion cell death, acute ischemic injury, infertility, wounds or other diseases connected with hPARP.

30. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is a hydrolase.

31. The use according to claim 30, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human Coagulation factor IX (Factor IX) or related molecules of the same structural class, preferably the peptide bonds between positions 21/22, 23/24, 36/37, 38/39, 59/60, 63/64, 74/75, 75/76, 87/88, 111/112, 112/113, 119/120, 127/128, 128/129, 129/130, 130/131, 136/137, 137/138, 149/150,151/152, 153/154, 162/163, 167/168, 173/174, 176/177, 190/191, 209/210, 222/223, 223/224 and/or 227/228, most preferred between positions 63/64, 127/128, 136/137, 149/150, 151/152, 173/174, 176/177 and/or 227/228 in Factor IX (SEQ ID NO: 166) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of hemophilia B or other diseases connected with Factor IX.

32. The use according to claim 30, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human caspase-2 (caspase-2) or related molecules of the same structural class, preferably the peptide bonds between positions 13/14, 14/15, 24/25, 28/29, 44/45, 45/46, 46/47, 48/49, 54/55, 65/66, 70/71, 81/82, 84/85, 96/97, 118/119, 120/121, 126/127, 154/155, 155/156, 186/187, 188/189, 212/213, 213/214, 227/228, 228/229, 247/248, 249/250, 251/252, 259/260 and/or 272/273, most preferred between positions 48/49, 81/82, 154/155, 186/187, 213/214, 228/229 and/or 251/252 in caspase-2 (SEQ ID NO: 169) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, pathological ischemic cell death, pathological reperfusion cell death, pathological retinal neuronal cell death, pathological apoptosis initiated by beta-amyloid toxicity or by trophic factor deprivation or other diseases connected with caspase-2.

33. The use according to claim 30, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human caspase-3 (caspase-3) or related molecules of the same structural class, preferably the peptide bonds between positions 12/13, 25/26, 29/30, 40/41, 47/48, 48/49, 51/52, 54/55, 56/57, 58/59, 66/67, 67/68, 73/74, 74/75, 77/78, 78/79, 79/80, 82/83, 83/84, 110/111, 139/140, 151/152, 152/153, 153/154, 158/159, 196/197, 198/199, 200/201, 201/202, 218/219, 220/221, 225/226 and/or 248/249, most preferred between positions 29/30, 40/41, 51/52, 56/57, 67/68, 73/74, 79/80, 83/84, 153/154, 218/219 and/or 225/226 in caspase-3 (SEQ ID NO: 170) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic acell death, reperfusion cell death, acute ischemic injury, infertility, wounds, neural degeneration in amyotrophic lateral sclerosis, Huntington´s, Infection with vesicular stomatitis virus (VSV) or other diseases connected with caspase-3.

34. The use according to claim 30, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human caspase-7 (caspase-7) or related molecules of the same structural class, preferably the peptide bonds between positions 35/36, 42/43, 43/44, 56/57, 57/58, 68/69, 72/73, 76/77, 79/80, 84/85, 88/89, 101/102, 102/103, 105/106, 106/107, 107/108, 149/150, 188/189, 189/190, 227/228, 228/229, 231/232, 232/233, 251/252, 255/256, 256/257 and/or 277/278, most preferred between positions 57/58, 79/80, 84/85, 102/103, 107/108, 228/229, 231/232, 232/233 and/or 255/256 in caspase-7 (SEQ ID NO: 171) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic acell death, reperfusion cell death, acute ischemic injury, infertility, wounds, toxin cell death induced by cytolethal distending toxin (CDT), acute lymphoblast leukemia or other diseases connected with caspase-7.

35. The use according to claim 30, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human caspase-9 (caspase-9) or related molecules of the same structural class, preferably the peptide bonds between positions 4/5, 19/20, 34/35, 35/36, 39/40, 49/50, 52/53, 53/54, 54/55, 63/64, 67/68, 71/72, 72/73, 79/80, 84/85, 112/113, 123/124, 151/152, 158/159, 215/216, 216/217, 217/218, 219/220, 223/224, 226/227, 229/230, 230/231, 233/234, 235/236 and/or 258/259, most preferred between positions 19/20, 35/36, 34/35, 52/53, 53/54, 71/72, 79/80, 219/220 and/or 230/231 in caspase-9 (SEQ ID NO: 172) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic acell death, reperfusion cell death, acute ischemic injury, infertility, wounds, neurological diseases like stroke, neurodegenerative diseases, brain injury caused by hypoxia, Parkinson's, amyotrophic lateral sclerosis (ALS) or other diseases connected with caspase-9.

36. The use according to claim 30, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human cathepsin B (cathepsin B) or related molecules of the same structural class, preferably the peptide bonds between positions 8/9, 9/10, 18/19, 53/54, 69/70, 75/76, 78/79,

85/86, 86/87, 94/95, 95/96, 124/125, 127/128, 130/131, 141/142, 146/147, 148/149, 151/152, 158/159, 159/160, 165/166, 166/167, 184/185, 194/195, 224/225, 227/228, 238/239, 245/246 and/or 252/253, most preferred between positions 75/76, 85/86, 95/96, 124/125, 130/131, 141/142, 148/149, 158/159 and/or 194/195 in cathepsin B (SEQ ID NO: 179) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of inflammatory bowel disease, Crohn´s disease, colitis ulcerosa or other diseases connected with cathepsin B.

37. The use according to claim 30, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human cathepsin L (cathepsin L) or related molecules of the same structural class, preferably the peptide bonds between positions 9/10, 10/11, 40/41, 41/42, 44/45, 72/73, 76/77, 79/80, 86/87, 87/88, 95/96, 96/97, 99/100, 103/104, 104/105, 114/115, 117/118, 120/121, 124/125, 141/142, 148/149, 155/156, 159/160, 160/161, 182/183, 189/190, 193/194, 198/199, 191/192, 192/193, 205/206 and/or 206/207 most preferred between positions 40/41, 87/88, 95/96, 103/104, 120/121, 141/142, 155/156, 159/160, 192/193 and/or 206/207 in cathepsin L (SEQ ID NO: 181) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of inflammatory bowel disease, Crohn´s disease, colitis ulcerosa or other diseases connected with cathepsin L.

38. The use according to claim 30, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human matrix metalloproteinase-14 (hMMP-14) or related molecules of the same structural class, preferably the peptide bonds between positions 12/13, 20/21, 26/27, 27/28, 34/35, 35/36, 38/39, 47/48, 49/50, 57/58, 50/51, 53/54, 55/56, 58/59, 62/63, 72/73, 82/83, 84/85, 113/114, 115/116, 116/117, 141/142, 152/153, 154/155, 156/157, 165/166 and/or 166/167, most preferred between positions 27/28, 38/39, 55/56, 57/58, 58/59, 82/83, 113/114, 116/117, 141/142 and/or 152/153 in hMMP-14 (SEQ ID NO: 185) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of cancer or other diseases connected with hMMP-14.

39. The use according to claim 30, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human cathepsin D (cathepsin D) or related molecules of the same structural class, preferably the peptide bonds between positions 10/11, 18/19, 47/48, 54/55, 58/59, 62/63, 63/64,

67/68, 69/70, 75/76, 86/87, 111/112, 112/113, 141/142, 158/159, 161/162, 172/173, 174/175, 189/190, 191/192, 192/193, 197/198, 202/203, 203/204, 214/215, 223/224, 224/225, 227/228, 242/243, 243/244, 245/246, 246/247, 249/250, 266/267, 281/282, 283/284, 284/285, 288/289, 289/290, 293/294, 299/300, 310/311 and/or 336/337, most preferred between positions 54/55, 62/63, 63/64, 112/113, 158/159, 174/175, 189/190, 197/198, 224/225, 242/243, 245/246, 266/267, 281/282, 288/289 and/or 299/300 in cathepsin D (SEQ ID NO: 180) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of inflammatory bowel disease, Crohn´s disease, colitis ulcerosa or other diseases connected with cathepsin D.

40. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is a structural protein.

41. The use according to claim 40, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human Galectin-3 (hGalectin-3) or related molecules of the same structural class, preferably the peptide bonds between positions 5/6, 16/17, 26/27, 31/32, 35/36, 52/53, 55/56, 56/57, 65/66, 68/69, 70/71, 71/72, 72/73, 73/74, 80/81, 83/84, 92/93, 97/98, 102/103, 111/112, 113/114, 114/115,126/127, 128/129 and/or 134/135 most preferred between positions 55/56, 65/66, 70/71, 102/103, 113/114 and/or 128/129 in hGalectin-3 (SEQ ID NO: 182) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of inflammatory bowel disease, Crohn´s disease, colitis ulcerosa or other diseases connected with hGalectin-3.

42. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is an activity modulating protein.

43. The use according to claim 42, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human apoptotic protease activating factor 1 (hApaf-1) or related molecules of the same structural class, preferably the peptide bonds between positions 6/7, 13/14, 14/15, 17/18, 18/19, 19/20, 24/25, 27/28, 32/33, 39/40, 40/41, 41/42, 44/45, 46/47, 62/63, 63/64, 64/65, 66/67, 80/81, 81/82 and/or 82/83, most preferred between positions 13/14, 14/15, 18/19, 41/42, 62/63 and/or 64/65 in hApaf-1 (SEQ ID NO: 173) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic acell death, reperfusion cell death, acute ischemic injury, infertility, wounds or other diseases connected with hApaf-1.

44. The use according to claim 42, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human BH3 interacting domain death agonist (hBID) or related molecules of the same structural class, preferably the peptide bonds between positions 6/7, 15/16, 32/33, 36/37, 37/38, 38/39, 53/54, 54/55, 56/57, 57/58, 58/59, 62/63, 65/66, 73/74, 77/78, 79/80, 101/102, 116/117, 120/121, 122/123, 123/124, 124/125, 134/135, 140/141, 142/143, 143/144, 145/146 and/or 170/171, most preferred between positions 36/37, 53/54, 57/58, 62/63, 65/66, 73/74 and/or 79/80 in hBID (SEQ ID NO: 174) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, degenerative disorders like neurodegenerative diseases, ischemic acell death, reperfusion cell death, acute ischemic injury, infertility, wounds or other diseases connected with hBID.

45. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is a DNA binding protein.

46. The use according to claim 45, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human Tumor protein p53 (hp53) or related molecules of the same structural class, preferably the peptide bonds between positions 8/9, 10/11, 14/15, 17/18, 27/28, 33/34, 53/54, 55/56, 63/64, 78/79, 81/82, 87/88, 88/89, 93/94, 105/106, 109/110, 112/113, 114/115, 115/116, 116/117, 131/132, 135/136, 155/156, 156/157, 166/167, 180/181, 187/188, 189/190, 190/191, 192/193, 193/194 and/or 194/195, most preferred between positions 14/15, 27/28, 53/54, 88/89, 114/115, 116/117, 131/132, 155/156, 190/191 and/or 194/195 in hp53 (SEQ ID NO: 176) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of apoptosis associated disorders like immunodeficiency diseases (AIDS/HIV), Alzheimers, senescence, any degenerative disorders (neurodegenerative diseases), ischemic and reperfusion cell

death, acute ischemic injury, infertility, wounds or other diseases connected with hp53.

47. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is a type-1 single pass transmembrane protein.

48. The use according to claim 47, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human interleukin-1 receptor type 1 (hIL-1R) or related molecules of the same structural class, preferably the peptide bonds between positions 35/36, 42/43, 43/44, 44/45, 46/47, 56/57, 61/62, 72/73, 82/83, 98/99, 132/133, 137/138, 140/141, 145/146, 146/147, 148/149, 153/154, 171/172, 172/173, 190/191, 202/203, 203/204, 205/206, 242/243, 245/246, 251/252, 252/253, 253/254, 254/255, 261/262, 262/263, 265/266, 271/272, 272/273, 283/284, 285/286, 287/288, 290/291 and/or 298/299, most preferred between positions 44/45, 46/47, 52/53, 61/62, 137/138, 148/149, 153/154, 171/172, 172/173, 203/204, 252/253, 253/254, 261/262, 271/272 and/or 290/291 of hIL-1R (SEQ ID NO: 150) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of asthma, inflammations, rheumatic disorders or other diseases connected with hIL-1R.

49. The use according to claim 47, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human interleukin-2 receptor beta chain (hIL-2Rb) or related molecules of the same structural class, preferably the peptide bonds between positions 38/39, 40/41, 41/42, 42/43, 43/44, 49/50, 71/72, 76/77, 81/82, 85/86, 86/87, 89/90, 91/92, 102/103, 105/106, 118/119, 134/135, 152/153, 153/154, 154/155, 161/162, 163/164, 165/166, 175/176, 194/195 and/or 197/198, most preferred between positions 38/39, 43/44, 81/82, 118/119, 134/135, 153/154, 161/162, 165/166 and/or 194/195 of hIL-2Rb (SEQ ID NO: 151) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of acute myeloid leukemia, inflammation, psoriasis or other diseases connected with hIL-2Rb.

50. The use according to claim 47, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human interleukin-4 receptor alpha chain (hIL-4Ra) or related molecules of the same structural class, preferably the peptide bonds between positions 22/23, 32/33, 45/46, 52/53, 66/67,

67/68, 87/88, 112/113, 125/126, 127/128, 129/130, 141/142, 143/144, 148/149, 150/151, 154/155, 156/157, 160/161, 167/168, 173/174, 175/176, 177/178, 183/184 and/or 189/190, most preferred between positions 52/53, 66/67, 112/113, 125/126, 143/144, 154/155 and/or 160/161 in hIL-4Ra (SEQ ID NO: 152) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of asthma and allergy or other diseases connected with hIL-4Ra.

51. The use according to claim 47, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human tumor necrosis factor receptor (hTNFR) or related molecules of the same structural class, preferably the peptide bonds between positions 40/41, 49/50, 51/52, 53/54, 54/55, 56/57, 68/69, 75/76, 77/78, 78/79, 79/80, 84/85, 91/92, 99/100, 100/101, 107/108, 109/110, 131/132, 132/133, 147/148, 149/150, 157/158, 158/159 and/or 161/162, most preferred between positions 40/41, 49/50, 54/55, 78/79, 84/85, 99/100, 107/108, 109/110, 132/133, 149/150 and/or 157/158 in hTNFR (SEQ ID NO: 153) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of asthma, Crohn´s disease, HIV infection, inflammation, psoriasis, rheumatoid arthritis or other diseases connected with hTNFR.

52. The use according to claim 47, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human fibroblast growth factor receptor 1 (hFGFR-1) or related molecules of the same structural class, preferably the peptide bonds between positions 15/16, 19/20, 22/23, 23/24, 24/25, 32/33, 49/50, 55/56, 56/57, 58/59, 60/61, 69/70, 70/71, 93/94, 95/96, 96/97, 110/111, 114/115, 134/135, 181/182, 182/183, 189/190, 194/195, 195/196 and/or 215/216, most preferred between positions 19/20, 24/25, 49/50, 55/56, 58/59, 60/61, 95/96, 96/97, 110/111, 181/182, 189/190, 195/196 and/or 215/216 in hFGFR-1 (SEQ ID NO: 158) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of cancer, angiogenesis or other diseases connected with hFGFR-1.

53. The use according to claim 47, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human fibroblast growth factor receptor 2 (hFGFR-2) or related molecules of the same structural class, preferably the peptide bonds between positions 9/10, 10/11, 14/15, 17/18, 18/19,

19/20, 32/33, 44/45, 50/51, 51/52, 53/54, 55/56, 62/63, 90/91, 91/92, 104/105, 105/106, 109/110, 127/128, 135/136, 149/150, 150/151, 175/176, 176/177, 177/178, 182/183, 189/190, 190/191 and/or 210/211, most preferred between positions 19/20, 53/54, 55/56, 91/92, 105/106, 149/150, 150/151, 175/176, 176/177, 182/183, 189/190 and/or 210/211 in hFGFR-2 (SEQ ID NO: 159) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of cancers like astrocytomas or other diseases connected with hFGFR-2.

54. The use according to claim 47, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human beta-amyloid (hbeta-amyloid) or related molecules of the same structural class, preferably the peptide bonds between positions 1/2, 3/4, 5/6, 7/8, 10/11, 11/12, 16/17, 19/20, 20/21, 22/23, 23/24 and/or 28/29, most preferred between positions 7/8, 10/11, 11/12, 16/17 and/or 23/24 in hbeta-amyloid (SEQ ID NO: 190) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of Alzheimer's disease or other diseases connected with hbeta-amyloid.

55. The use according to claim 47, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human P-selectin (hP-selectin) or related molecules of the same structural class, preferably the peptide bonds between positions 8/9, 16/17, 17/18, 18/19, 22/23, 23/24, 36/37, 37/38, 40/41, 44/45, 45/46, 54/55, 55/56, 66/67, 67/68, 72/73, 74/75, 78/79, 80/81, 84/85, 85/86, 88/89, 92/93, 94/95, 96/97, 106/107, 107/108, 111/112, 112/113, 124/125, 129/130, 140/141, 152/153 and/or 154/155, most preferred between positions 17/18, 22/23, 44/45, 55/56, 72/73, 78/79, 84/85, 85/86, 107/108, 112/113, 152/153 and/or 154/155 in hP-selectin (SEQ ID NO: 177) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of inflammation or other diseases connected with hP-selectin.

56. The use according to claim 47, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human receptor tyrosine-protein kinase erbB-2 (hHER2) or related molecules of the same structural class, preferably the peptide bonds between positions 18/19, 70/71, 87/88, 88/89, 99/100, 116/117, 135/136, 143/144, 153/154, 163/164, 168/169, 188/189, 206/207, 216/217, 226/227, 252/253, 255/256, 258/259, 264/265, 266/267,

279/280, 311/312, 314/315, 318/319, 326/327, 330/331, 332/333, 357/358, 360/361, 373/374, 395/396, 477/478, 479/480, 480/481, 481/482, 485/486, 495/496, 514/515, 520/521, 521/522, 523/524, 530/531, 532/533, 536/537, 558/559, 560/561, 568/569, 577/578, 592/593, 597/598 and/or 598/599, most preferred between positions 88/89, 135/136, 143/144, 226/227, 252/253, 255/256, 258/259, 314/315, 318/319, 360/361, 480/481, 485/486, 495/496, 520/521, 523/524, 560/561, 568/569, 577/578 and/or 592/593 in hHER2 (SEQ ID NO: 183) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of cancer or other diseases connected with hHER2.

57. The use according to claim 47, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human Tumor necrosis factor receptor superfamily member 14 (hvemA) or related molecules of the same structural class, preferably the peptide bonds between positions 14/15, 18/19, 23/24, 24/25, 26/27, 31/32, 54/55, 62/63, 68/69, 71/72, 75/76, 95/96, 101/102 and/or 103/104, most preferred between positions 23/24, 26/27, 62/63, 68/69, 95/96, 101/102 and/or 103/104 in hvemA (SEQ ID NO: 191) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of inflammatory bowel disease, Crohn´s disease, colitis ulcerosa or other diseases connected with hvemA.

58. The use according to any one of claims 1 to 12, wherein the target substrate hydrolyzed by the protease is a multipass transmemebrane protein.

59. The use according to claim 58, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human C-X-C chemokine receptor type 5 (hCCR5) or related molecules of the same structural class, preferably the peptide bonds between positions 8/9, 10/11, 12/13, 16/17, 19/20, 22/23, 23/24, 25/26, 27/28, 29/30, 34/35, 42/43, 50/51, 110/111, 115/116, 120/121, 123/124, 189/190, 201/202, 204/205, 207/208, 211/212, 215/216, 216/217, 219/220, 281/282, 285/286, 287/288, 290/291 and/or 294/295 in hCCR5 (SEQ ID NO: 154) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of HIV infection or other diseases connected with hCCR5.

60. The use according to claim 58, wherein

(i) the protease is capable of hydrolyzing peptide bonds in human C-X-C chemokine receptor type 3 (hCXCR3) or related molecules of the same structural class, preferably the peptide bonds between positions 4/5, 7/8, 13/14, 21/22, 23/24, 27/28, 28/29, 29/30, 35/36, 46/47, 47/48, 52/53, 53/54, 112/113, 117/118, 119/120, 125/126, 195/196, 197/198, 205/206, 207/208, 212/213, 278/279, 282/283, 288/289, 292/293, 293/294, 295/296 and/or 297/298 in hCXCR3 (SEQ ID NO: 155) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of multiple sclerosis, rheumatoid arthritis or other diseases connected with hCXCR3.

61. The use according to claim 58, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human C-C chemokine receptor type 1 (hCCR1) or related molecules of the same structural class, preferably the peptide bonds between positions 2/3, 8/9, 9/10, 10/11, 11/12, 15/16, 17/18, 18/19, 26/27, 29/30, 30/31, 32/33, 92/93, 93/94, 94/95, 96/97, 97/98, 98/99, 99/100, 101/102, 103/104, 107/108, 173/174, 176/177, 177/178, 178/179, 187/188, 190/191, 193/194, 194/195, 195/196, 196/197, 266/267, 272/273, 274/275, 277/278 and/or 280/281 in hCCR1 (SEQ ID NO: 160) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of multiple sclerosis or other diseases connected with hCCR1.

62. The use according to claim 58, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human C-C chemokine receptor type 2 (hCCR2) or related molecules of the same structural class, preferably the peptide bonds between positions 6/7, 8/9, 9/10, 11/12, 15/16, 18/19, 19/20, 23/24, 25/26, 27/28, 34/35, 36/37, 38/39, 105/106, 106/107, 108/109, 114/115, 180/181, 183/184, 184/185, 185/186, 188/189, 193/194, 194/195, 196/197, 198/199, 201/202, 206/207, 270/271, 271/272, 272/273, 278/279 and/or 284/285 in hCCR2 (SEQ ID NO: 161) or between analogous positions in related molecules; and/or
(ii) the medicament is suitable for the treatment of multiple sclerosis, rheumatoid arthritis or other diseases connected with hCCR2.

63. The use according to claim 58, wherein
(i) the protease is capable of hydrolyzing peptide bonds in human C-X-C chemokine receptor type 4 (hCCR-4) or related molecules of the same structural class,

preferably the peptide bonds between positions 7/8, 10/11, 12/13, 14/15, 15/16, 20/21, 21/22, 22/23, 25/26, 26/27, 30/31, 31/32, 32/33, 36/37, 38/39, 102/103, 103/104, 104/105, 107/108, 110/111, 181/182, 182/183, 183/184, 184/185, 187/188, 188/189, 190/191, 193/194, 195/196, 262/263, 268/269, 271/272, 275/276, 277/278, 282/283 and/or 283/284 in hCCR-4 (SEQ ID NO: 189) or between analogous positions in related molecules; and/or

(ii) the medicament is suitable for the treatment of inflammation or other diseases connected with hCCR-4.

64. The use according to any one of claims 1 to 12, wherein the target protein is a member of the group consisting of a5B1 (VLA-5), ADAM-12-S, ADAM-9, Adiponectin, ADP receptor P2Y(12), ADP receptor P2T(AC), ADP receptor P2Y(1), advanced glycation endproducts receptor (RAGE), Aldose reductase, angiotensin-converting enzyme (ACE), Anthrax EF: Edema Factor, Anthrax LF: Leathal Factor, Anthrax PA: Protective Antigen, AP-1, B7-1, B7-2, CD28, BAD, BAX, Bcl-2, BCR-Abl, beta-catenin, beta-lactamase from Moraxella catarrhalis, beta-lactamase from Pseudomonas aeruginosa, human GPI-anchored PrP(C) , swine GPI-anchored PrP(C) , sheep GPI-anchored PrP(C), bovine GPI-anchored PrP(C), human PrP(Sc), swine PrP(Sc), sheep PrP(Sc), bovine PrP(Sc), C5/C5a, NodH sulfotransferase, UDP-glucuronosyltransferase, Heparan sulfate 3-o-sulfotransferase isoform 3, human estrogen sulfotransferase, phenol sulfotransferase SULT1A1 (ST1A3), human GalCer sulfotransferase, caspase-6, caspase-1, caspase-8, , CCR8, CD18, CD3, CD4, CD40, CD30L, CD30L receptor, CD33, CD35, CD40L, CD46 (MCP), CD52, CD55 (decay accelerating factor), CD59, cdk-4, chitin from fungal pathogens, CINC/GRO-alpha, c-Jun, ClfA protein, c-met (Hepatocyte growth factor receptor), CO-029, human tetraspanin-1, tetraspanin-2, tetraspanin-3, tetraspanin-4, tetraspanin-5, tetraspanin-6, tetraspanin TM4-B, Corticotropin-releasing hormone (CRH), CTLA-4 (CD152), CXCR1, CXCR2, cyclo-oxygenase (COX), cytocrome C, diacylglycerol acyltransferase (DGAT), ErbB3 (Her-3), ErbB4 (Her4), EGFR endodomain (intracellular), eotaxin, EPA1, EGP-2, ERK, E-Selectin, exfoliative toxin, gluten exorphin A5, gluten exorphin B4, gluten exorphin B5, gluten exorphin C, alpha-casein exorphin on CA1, F protein from HIV, factor Xa, fibrinogen, G(q/11), ganglioside GT3, ganglioside GD3, ganglioside GM-1, ganglioside GM2-1, glycogen phosphorylase (GP), GM-CSF, Gp41 from HIV, Hag, hemaglutinin, Heme Oxygenase, HIF I, Histone deacetylase, IgE, IgE-receptor, IGF, IL-1, IL-12-alpha, IL-12R, IL-13R, IL-15, IL-15R, IL-18R, IL27, IL-2R-beta, IL-2R-alpha, IL31, IL-5R,

IL-7, IL-9, inner layer protein p24, Integrin a(4), Integrin a(4) b(1), Integrin a(4) b(7), Integrin a(v) b(3), Integrin b(1), Integrin b(7), IL-11, IP-10, Mig, MIP-1alpha, IRAK-1, IRAK-4, Jun N-terminal kinase (JNK), Kallikrein, leukocyte function-associated antigen-1, leukotriene B(4), leukotriene D4 (LTD4), leukotriene receptor Cys-LT1, leukotriene receptor Cys-LT2, leukotriene receptor LTB4-1, leukotriene receptor LTB4-2, Lewis y/b antigen, lipoprotein(a), LT-alpha, lyphotoxin beta, matrix metalloprotease-1 (MMP-1), mcaP adherence protein, MCP-1, M-CSF, MDC, MHC class II receptor, MID, MMP-12, MMP-13, MN antigen, muscarinic receptor M1, muscarinic receptor M3, NAD(P)H oxidase, neutrophil elastase, NF-kappaB, nucleocapsid p17 from HIV, p10 protease from HIV, p115-RhoGEF A-site, p32 integrase from HIV, p64 Reverse transcriptase from HIV, PAF, parathyroid hormone, parathyroid hormone-related peptide (PTHrP) receptor, Platelet cyclic adenosine monophosphate (cAMP) phosphodiesterase, phosphodiesterase 4, Polymorphic epithelial mucin (PEM), porin F (OprF) from Pseudomonas aeruginosa, Proteasome, Protein-Tyrosine Phosphatase PTPase 1B (PTP1B), PTH receptor, RANK, RANKL, Rip2, RSV (respiratory syncytium virus) fusion protein, Sortase from Streptococcus mutans, Src-Homology Inositol Phosphatase-2 (SHIP2), T1/ST2, TARC, TGF-beta-1, TGF-beta-2, TGF-beta-3, TGF-beta-4, TGF-betaRI , thrombin, tissue factor/factor VIIa, Toll-like receptor-1, Toll-like receptor-2, Toll-like receptor-3, Toll-like receptor-4, Toll-like receptor-5, Toll-like receptor-6, Toll-like receptor-7, Toll-like receptor-8, Toll-like receptor-9, Toll-like receptor-10, transmembrane PTPase leukocyte antigen-related (LAR), triggering receptor expressed on myeloid cells (TREM-1), UspA1, VAP-1 (Vascular adhesion protein-1), VEGFR-3, Wnt protein 2, Wnt protein 3, Wnt protein 4, Wnt protein 7B, OSM-receptor, IL-6-receptor alpha chain, IL-6-receptor beta chain, Lymphotoxin-beta receptor and Leukemia inhibitory factor receptor.

65. The use according to any one of claims 1 to 64, wherein the protease comprises
(i) at least one further proteinacious component, preferably being selected from the group consisting of binding domains, receptors, antibodies, regulation domains, pro-sequences, and fragments thereof, and/or
(ii) at least one further functional component, preferably being selected from the group consisting of polyethylenglycols, carbohydrates, lipids, fatty acids, nucleic acids, metals, metal chelates, and fragments or derivatives thereof.

66. Use of an enzyme according to any one of claims 1 to 65 for *in vivo* or *in vitro* diagnostic purposes.

67. A pharmaceutical or diagnostic composition comprising one or more enzymes according to any one of claims 1 to 65, said pharmaceutical or diagnostic composition optionally comprising pharmaceutically or diagnostically acceptable carrier(s), excipient(s) and/or auxiliary agent(s).

68. A method for cleaving a target substrate as defined in claims 13 to 64 *in vivo* or *in vitro*, which comprises contacting the target substrate with a protease as defined in claims 1 to 65.

69. A method for treatment of a disease in a patient connected with a specific target substrate as defined in anyone of claims 13 to 64, which comprises administering the patent a suitable amount of a protease with defined specificity for said specific target substrate as defined in anyone of claims 1 to 65.

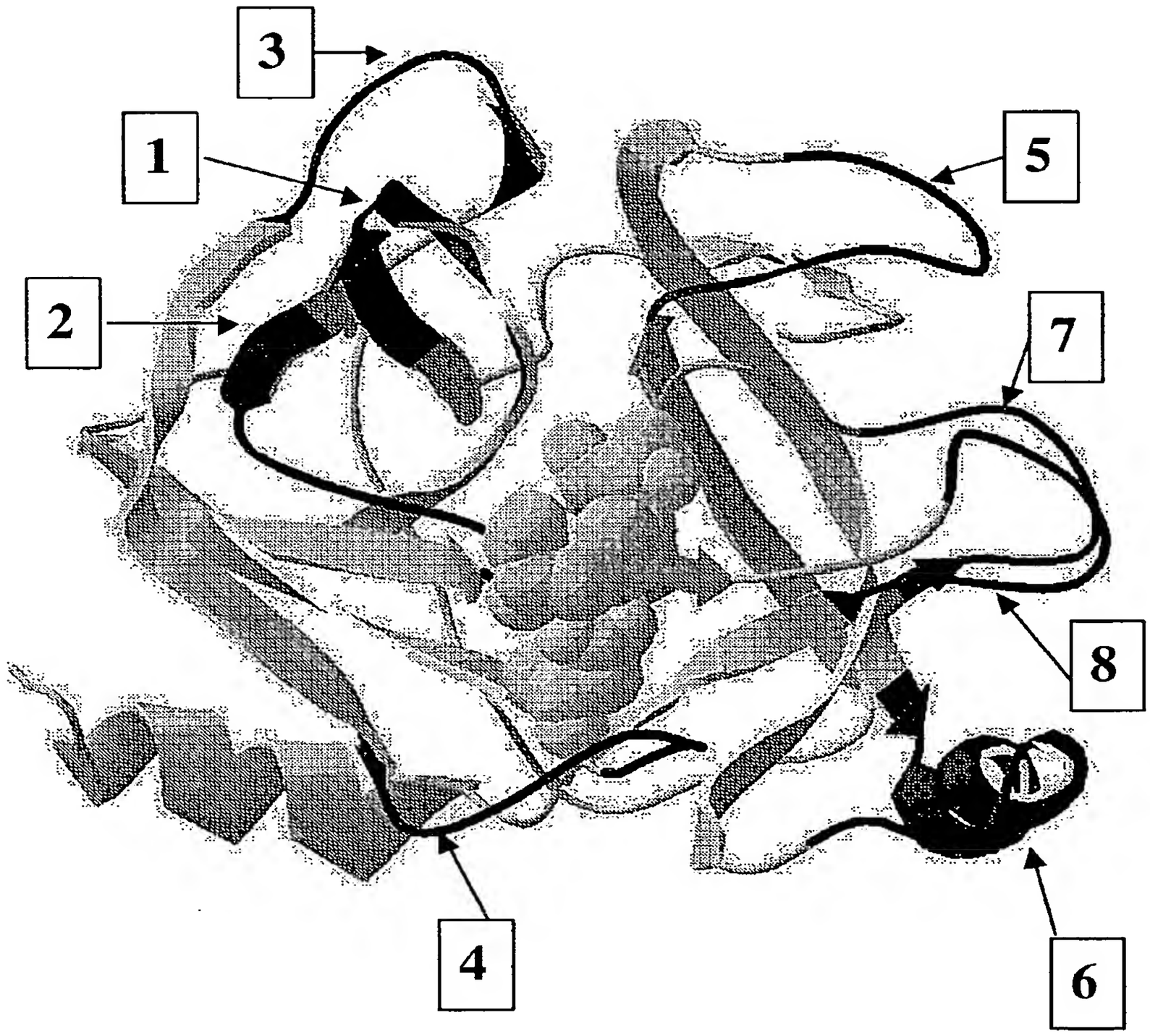

Fig. 1

```
Trypsin           IVGGYNCEENSVPYQVSL---NSGYHF-CGGSLINEQWVVSAGHCY----
a-Thrombin        IVEGSDAEIGMSPWQVMLFRKSPQELL-CGASLISDRWVLTAAHCLLYPP
Enteropeptidase   IVGGSNAKEGAWPWVVGL---YYGGRLLCGASLVSSDWLVSAAHCVYGRN
                  ** *         *  * *           ** **    *   * ** ----

Trypsin           ------KSRIQVRLGEH---NIEVLEGN-EQFINAAKIIRHPQYD-RKTL
a-Thrombin        WDKNFTENDLLVRIGKH---SRTRYERNIEKISMLEKIYIHPRYNWRENL
Enteropeptidase   LE----PSKWTAILGLHMKSNLTSPQTV-PRLID--EIVINPHYN-RRRK
                  -1----         * *                      *   * * *

Trypsin           NNDIMLIKLSSRAVINARVSTISLPTA----PPAT-----GTKCLISGWG
a-Thrombin        DRDIALMKLKKPVAFSDYIHPVCLPDR----ETAASLLQAGYKGRVTGWG
Enteropeptidase   DNDIAMMHLEFKVNYTDYIQPICLPEENQVFPP-------GRNCSIAGWG
                    **    *               **  ------2------*      ***

Trypsin           N-----TASSGADYPDELQCLDAPVLSQAKCEASYPG-KITSNMFCVGFL
a-Thrombin        NLKETWTANVGKGQPSVLQVVNLPIVERPVCKDSTRI-RITDNMFCAGYK
Enteropeptidase   T-----VVYQGTT-ANILQEADVPLLSNERCQQQMPEYNITENMICAGYE
                   --3--    *      **    *       *         ** ** * *

Trypsin           -EGGK--DSCQGDSGGPVVCNGQ----LQ------GVVSWGDGCAQKNKP
a-Thrombin        PDEGKRGDACEGDSGGPFVMKSP----FNNRWYQMGIVSWGEGCDRDGKY
Enteropeptidase   -EGGI--DSCQGDSGGPLMCQENNRWFLA------GVTSFGYKCALPNRP
                     *    * * ******      ------4-----*  * *  *

Trypsin           GVYTKVYNYVKWIKNTIAANS-
a-Thrombin        GFYTHVFRLKKWIQKVIDQFGE
Enteropeptidase   GVYARVSRFTEWIQSFLH----
                  * *  *     **
```

Fig. 2

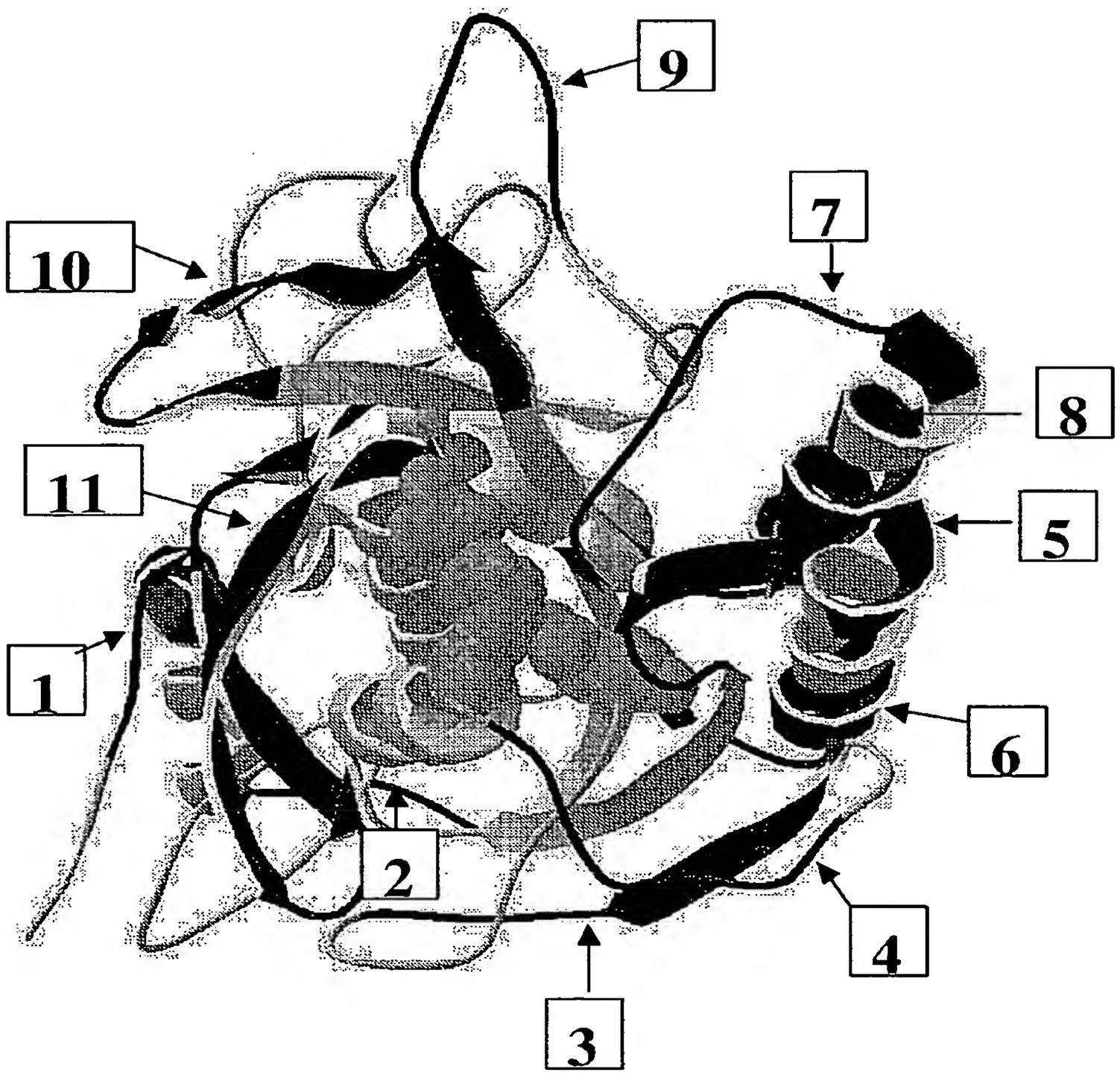

Fig. 3

```
sub       IAHEYAQSV--PY---------------GISQ--IKAPALHSQGY---------------
furin     VAKRRAKRD--VYQEPTDPKFPQQWYLSGVTQRDLNVKEAWAQGF---------------
PC_SK1    EKERSKRSALRDS---------------ALNL--FNDPMWNQQWYLQDTRMTAALPKLDL
PC_SK5    NTHPCQ----------------------SD--MNIEGAWKRGY---------------
                 ------------1---------------      ---------------2--

sub       ----------TGSNVKVAVIDSGIDSSHPDL--NVRGGAS--FVPSETN-------P---
furin     ----------TGHGIVVSILDDGIEKNHPDLAGNYDPGAS--FDVNDQD-------PDPQ
PC_SK1    HVIPVWQKGITGKGVVITVLDDGLEWNHTDIYANYDPEASYDFNDNDHD-------P---
PC_SK5    ----------TGKNIVVTILDDGIERTHPDL---MQNYDA--LASCDVNGNDLDPMP---
          ------------        * *    * *------3------   --------------

sub       ----YQ-----DGSS---HGTHVAGTIA--AL-NNSIGVLGVSPSASLYAVKVLDS----
furin     PRYTQM-----NDNR---HGTRCAGEVA--AVANNGVCGVGVAYNARIGGVRMLD-----
PC_SK1    ----FPRYDPTNENK---HGTRCAGEIAMQAN-NHKCGV-GVAYNSKVGGIRMLDG----
PC_SK5    ----RY-----DASNENKHGTRCAGEVA--AAANNSHCTVGIAFNAKIGGVRMLDGDVTD
          ------4----------- **  **  *  *  *      *              -------

sub       -TGSGQYSWIINGIE-WAISNNMDVINMSLG---------GPT--GSTA------LKT--
furin     ----GEVTDAVEARS-LGLNPNHIHIYSASW---------GPEDDGKTVDGPARLAEE--
PC_SK1    -IVTDAIEASSIGFN----PGHVDIYSASWGPNDDGKTVEGP---GRLA------QKAFE
PC_SK5    MVEAKSVSFNPQHVHIYSASWGPDDDGKTVD---------GPA--PLT--------RQ--
          -5-------     -----6----   ----------7--------- ----------8-

sub       --VVDKAVSSG-----IVVAAAAGNEGSS-------GSTSTVGYPAKYPSTIAVGAV---
furin     --AFFRGVSQGRGGLGSIFVWASGNGGREHDSCNCDGYTNSI-YTLSISSATQFGNV---
PC_SK1    YGVKQGRQGKG-----SIFVWASGNGGRQ-------GDNCDCD---GYTDSIYTISI---
PC_SK5    --AFENGVRMGRRGLGSVFVWASGNGGRSKDHCSCDGYTNSI-YTISISSTAESGKKPWY
          -------8----------  *----------9--------                ----

sub       --N------SSNQR--------------ASFSSAG-SELDVMAPGVSIQSTLPGGTYGAY
furin     --PWYSEACSSTLA--------------TIYSSGNQNEKQIVTTDLRQKCT------ESH
PC_SK1    --S------SASQQGLSPWYAEKCSSTLATSYSSG-DYTDQRITSADLHNDCT----ETH
PC_SK5    LEE------CSSTL--------------ATTYSSG-ESYDKKI----ITTDLRQRCTDNH
          ----------10--------------------*        ---------------11---

sub       NGTSMATPHVAGAAAALIL--SKHP--TWTNAQVRDRLESTATY--LG-NSFYYGKGLINV
furin     TGTSASAPLAAGIIALTLEANKNL--TWRDMQHLVVQTSKPAH--LN-ADDWATNGVGRK
PC_SK1    TGTSASAPLAAGIFALAL--EANP--NLTWRDMQHLVVWTSEYDPLA-NNPGWKKNGAGL
PC_SK5    TGTSASAPMAAGIIALAL--EANPFLTWRDVQHVIVRTSRAGH--LNANDWKTNAAGFKV
          ---*    *  **  ** *                                  *
```

Fig. 4

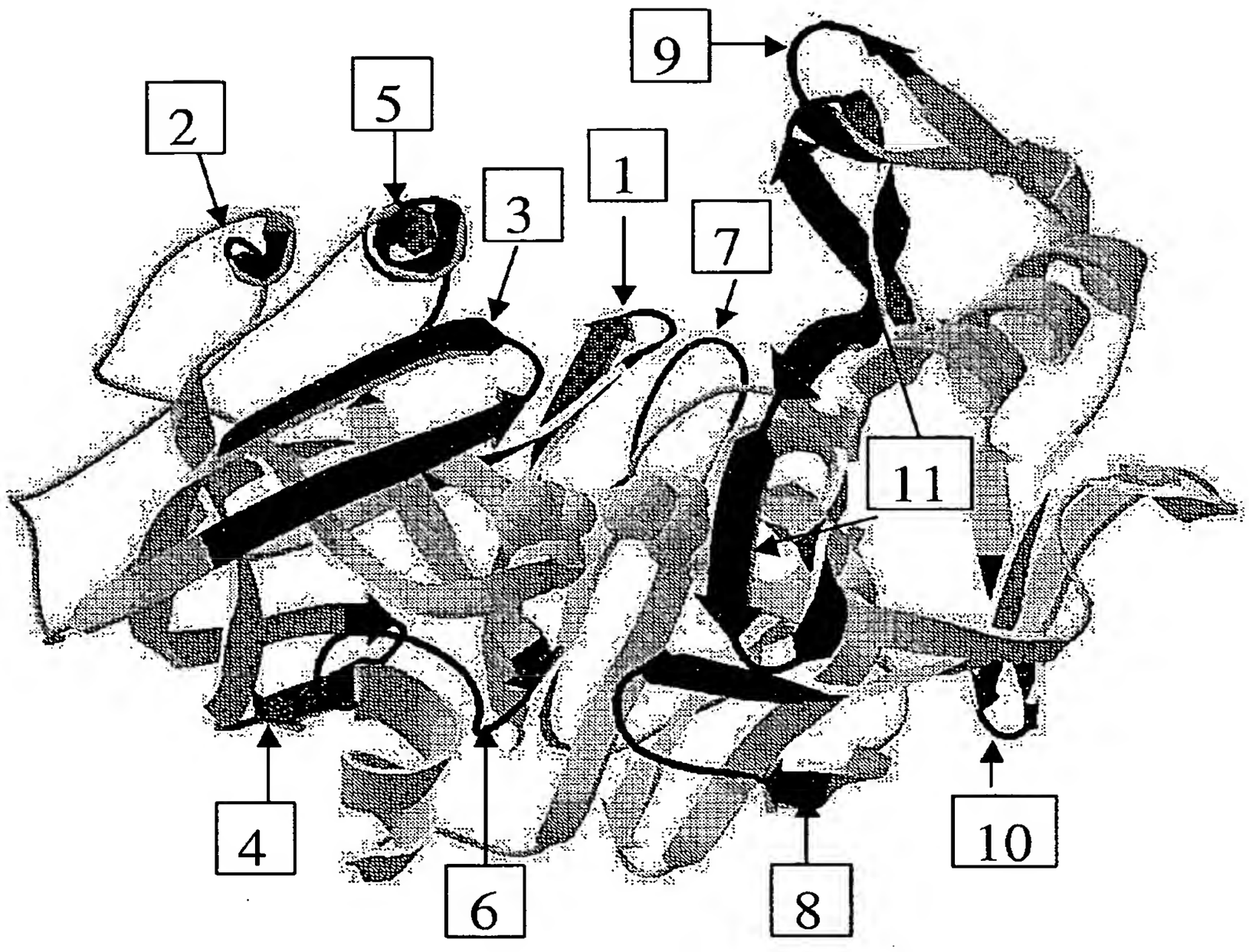

Fig.5

```
Peps.     TLVDEQP----LENYLDMEYFGTIGIGTPAQDFTVVFDTGSSNLWVPSVYCSSL--ACTN
Secr.     EMVDN------LRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFAVGAAPHPFL------
Cath.     PAVTEGPIPEVLKNYMDAQYYGEIGIGTPPQCFTVVFDTGSSNLWVPSIHCKLLDIACWI
            * ----1-----     *      * * *      ******  *      -----2--

Peps.     HNRFNPEDSSTYQSTSETVSITYGTGSMTGILGYDTVQV-----------G---GISDTN
Secr.     HRYYQRQLSSTYRDLRKGVYVPYTQGKWEGELGTDLVSI-----------PHGPNVTVRA
Cath.     HHKYNSDKSSTYVKNGTSFDIHYGSGSLSGYLSQDTVSVPCQSASSASALG---GVKVER
          -       ****       -----3----* *   * ----------4-----------

Peps.     QIFGLSETEPGSFLYYAPFDGILGLAYPSIS--SSGATPVFDNIWNQGLVSQDLFSVYLS
Secr.     NIAAITESDK-FFINGSNWEGILGLAYAEIARPDDSLEPFFDSLVKQTHVP-NLFSLQLC
Cath.     QVFGEATKQPGITFIAAKFDGILGMAYPRIS--VNNVLPVFDNLMQQKLVDQNIFSFYLS
                    ----5----**** ------6-------- **    *  *    **  *

Peps.     ADD----------KS--GSVVIFGGIDSSYYTGSLNWVPVTVEGYWQITVDSITMNGETI
Secr.     GAGFPLNQSEVLASV--GGSMIIGGIDHSLYTGSLWYTPIRREWYYEVIIVRVEINGQDL
Cath.     RDP----------DAQPGGELMLGGTDSKYYKGSLSYLNVTRKAYWQVHLDQVEVASGLT
          --------7---------     ** *   * ***  -----8-----

Peps.     A--CAEGC--QAIVDTGTSLLTGPTSPIANIQSDIGASENSD--------GDMVVSCSAI
Secr.     KMDCKEYNYDKSIVDSGTTNLRLPKKVFEAAVKSIKAASSTEKFPDGFWLGEQLV-CWQA
Cath.     L--CKEGC--EAIVDTGTSLMVGPVDEVRELQKAIGAVPLIQ--------GEYMIPCEKV
             * *      *** **     *          * *  -------9------ *

Peps.     SSLPDIVFTI-----------NGVQYPVPPSAYILQSEGS----CISGFQGMNVP-TESG
Secr.     GITPWNIFPVISLYLMGEVTNQSFRITILPQQYLRPVEDV----ATSQDDCYKFAISQSS
Cath.     STLPAITLKL-----------GGKGYKLSPEDYTLKVSQAGKTLCLSGFMGMDIP-PPSG
             *   -------10-------    *  * --------------11-----------

Peps.     ELWILGDVFIRQYFTVFDRANNQVGLAPVA
Secr.     TGTVMGAVIMEGFYVVFDRARKRIGFAVSA
Cath.     PLWILGDVFIGRYYTVFDRDNNRVGFAEAA
          --   * *       ****     * *  *
```

Fig. 6

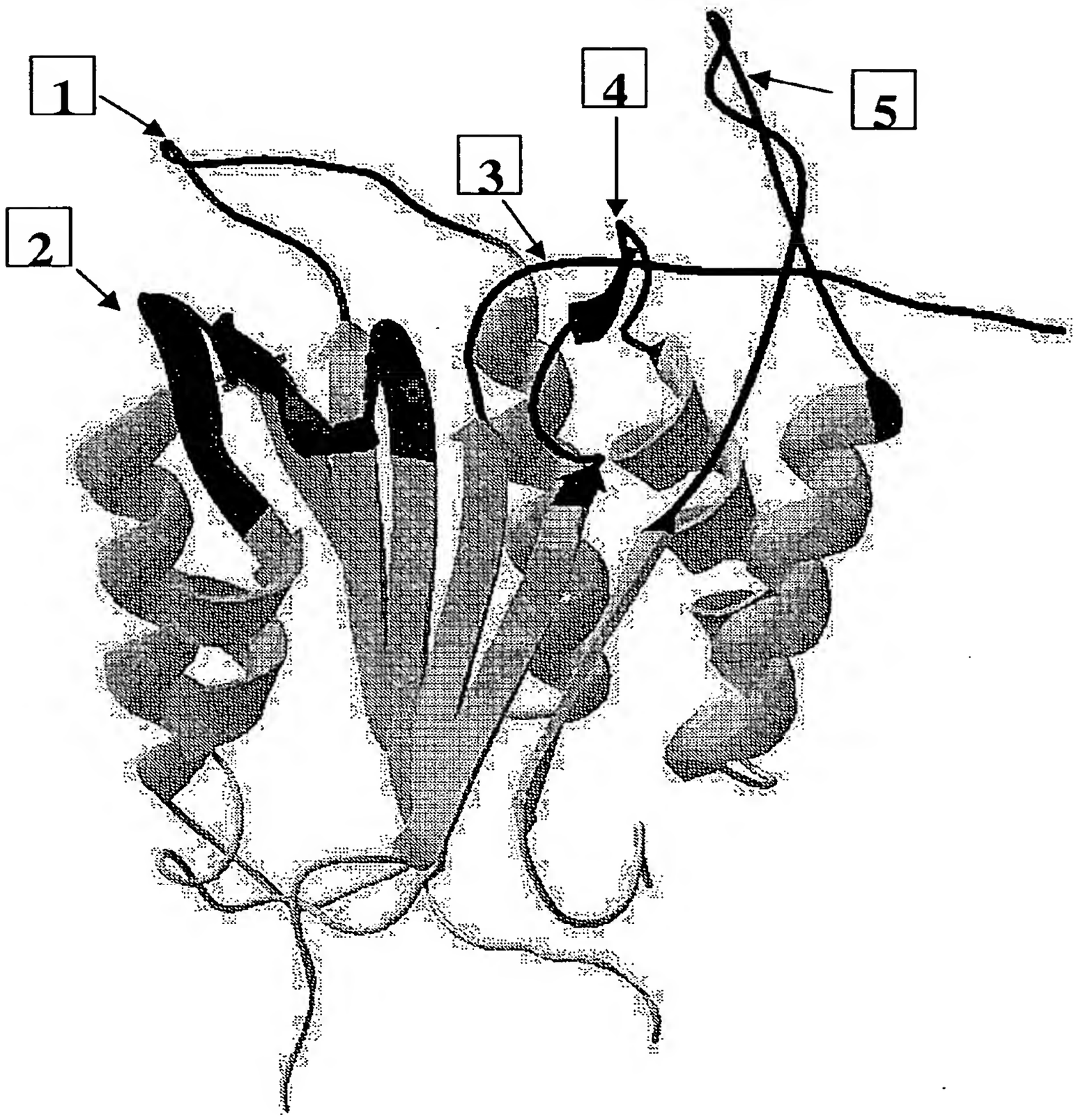

Fig. 7

```
01   MLEADDQGCI EEQGVEDSAN EDSVDAKPDR SSFVPSLFSK KKKNVIMRSI KTTRDRVPIY

61   QYNMNFEKLG KCIIINNKNF DKVTGMGVRN GTDKDAEALF KCFRSLGFDV IVYNDCSCAK
                          ------1---------

121  MQDLLKKASE EDHTNAACFA CILLSHGEEN VIYGKDGVTP IKDLTAHFRG DRSKTLLEKP
                                --------2---------

181  KLFFIQACRG TELDDGIQAD SGPINDTDAN·PRYKIPVEAD FLFAYSTVPG YYSWRSPGRG
          -----3--------                                ------------4-----

241  SWFVQALCSI LEEHGKDLEI MQILTRVNDR VARHFESQSD DPHFHEKKQI PCVVSMLTKE
     ---                                          ----------5------------

301  LYFSQ
```

Fig. 8

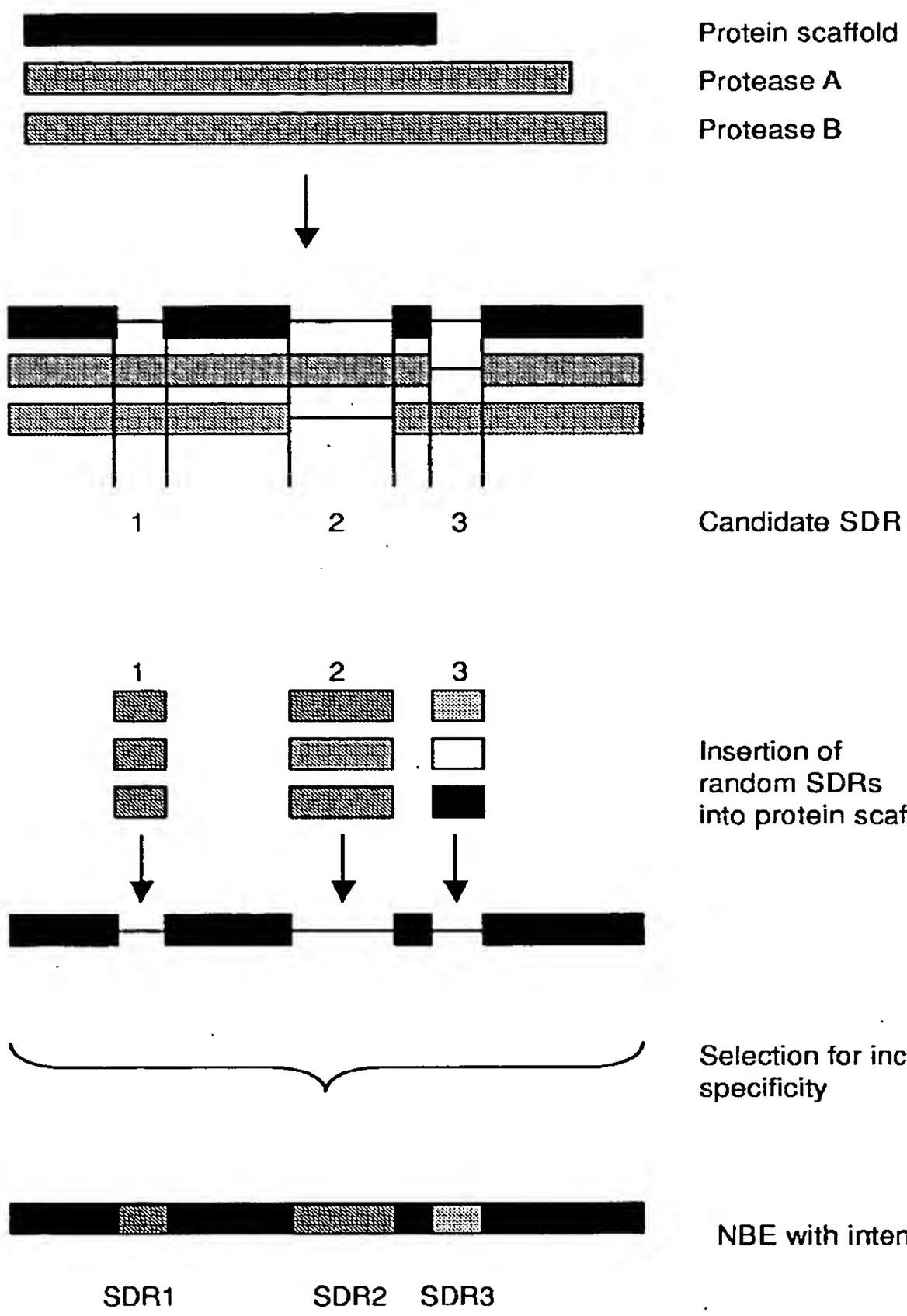
Protein scaffold
Protease A
Protease B
1
2
3
Candidate SDR insertion sites
1
2
3
Insertion of
random SDRs
into protein scaffold
Selection for increased
specificity
SDR1
SDR2
SDR3
NBE with intended specificity

Fig. 9

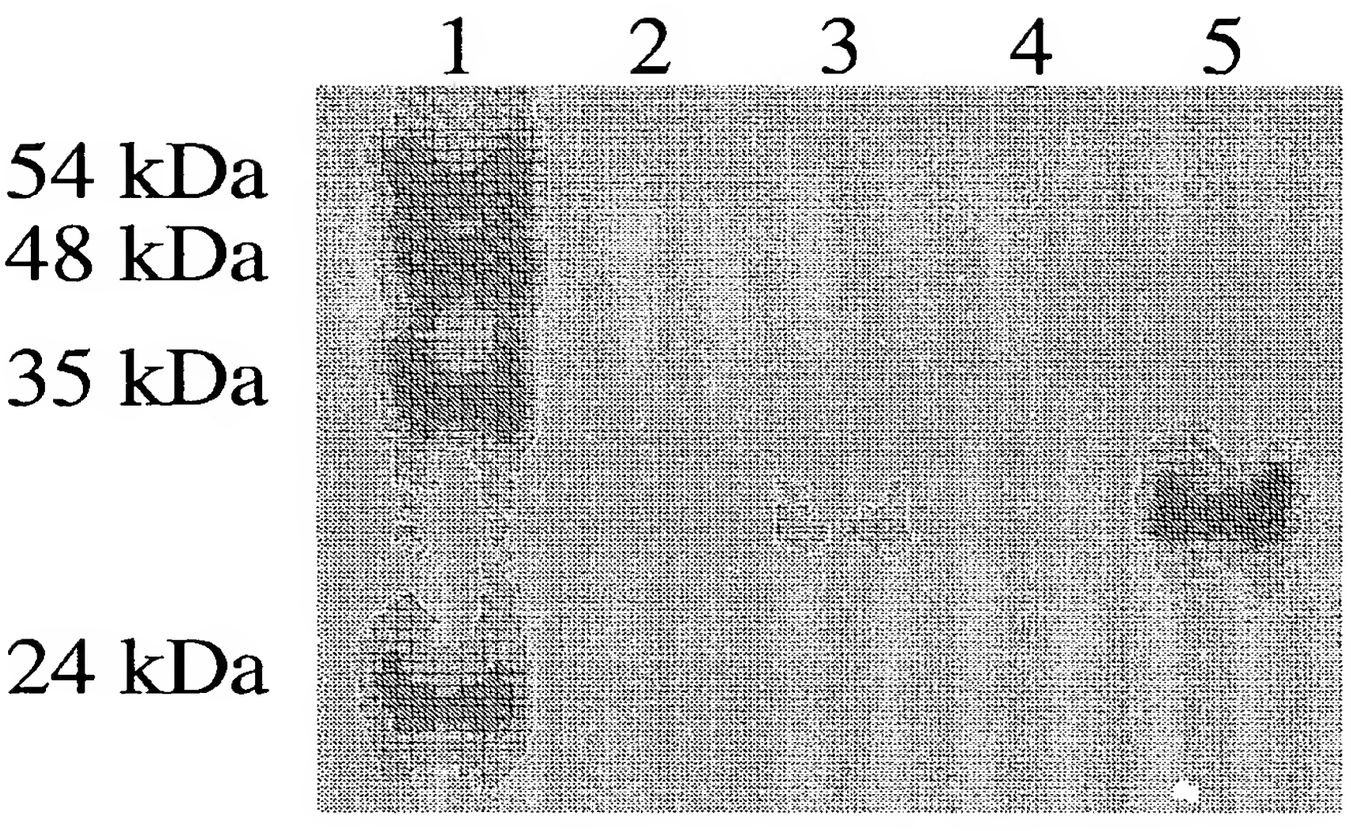

Fig. 10

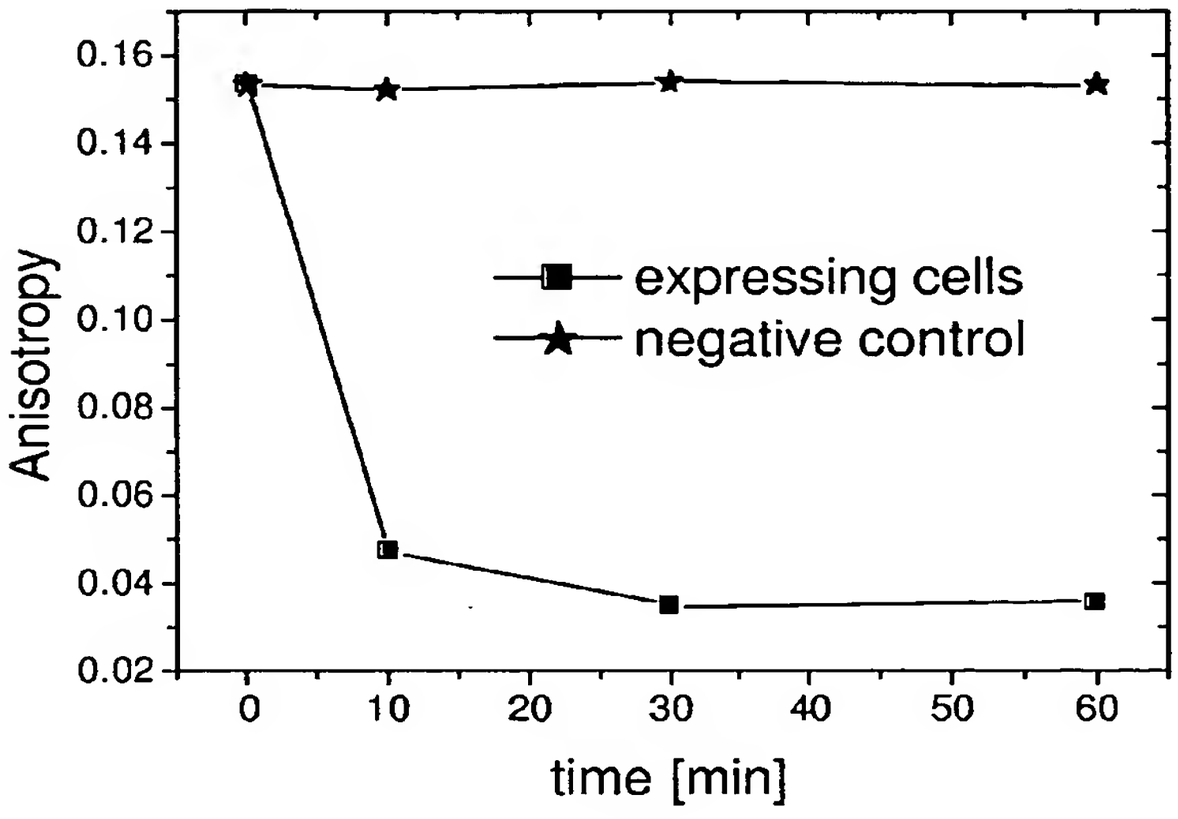
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
Anisotropy
0
10
20
30
40
50
60
time [min]
expressing cells
negative control

Fig. 11

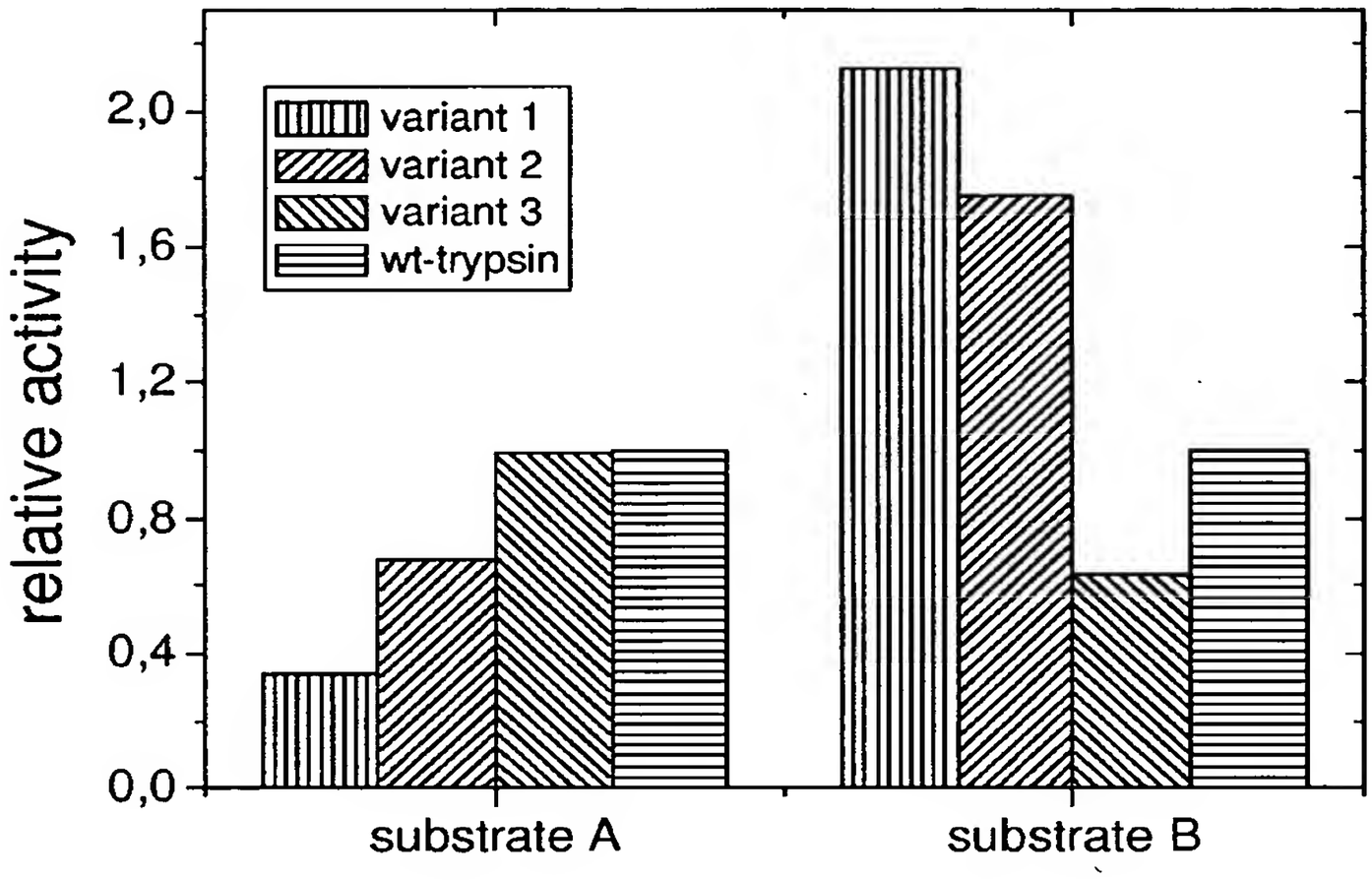
relative activity
2,0
1,6
1,2
0,8
0,4
0,0
variant 1
variant 2
variant 3
wt-trypsin
substrate A
substrate B

Fig. 12

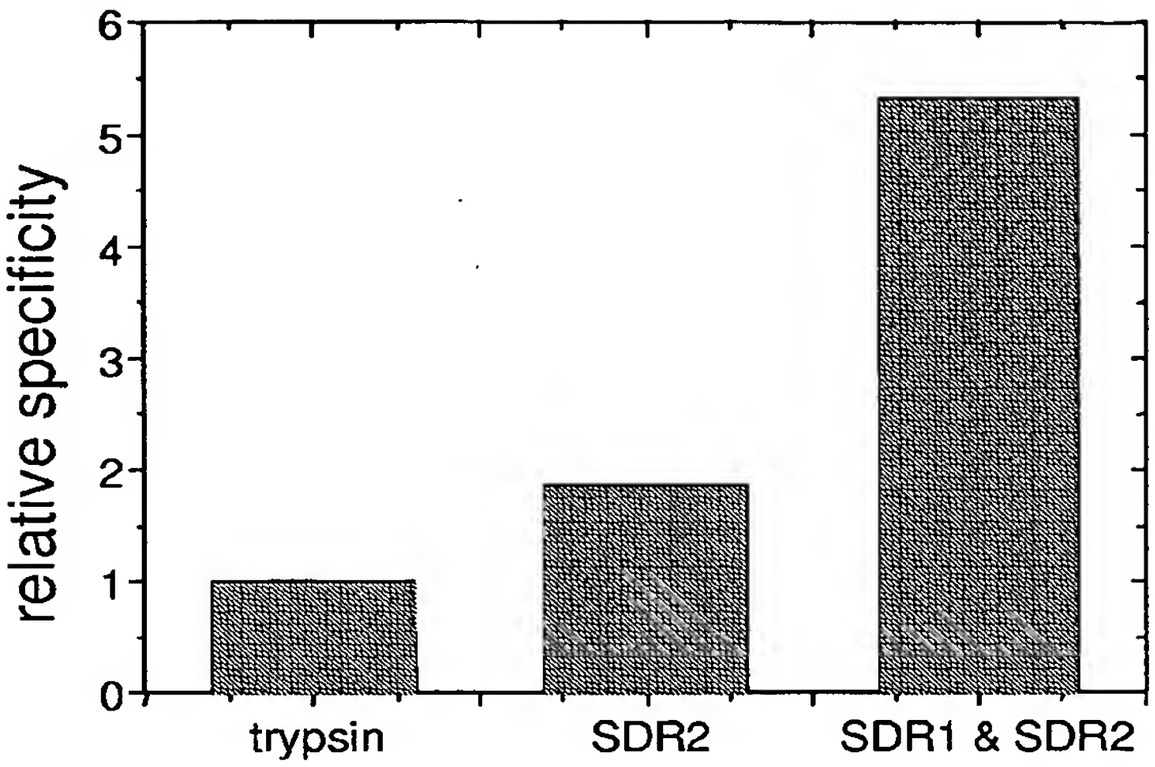

Fig. 13

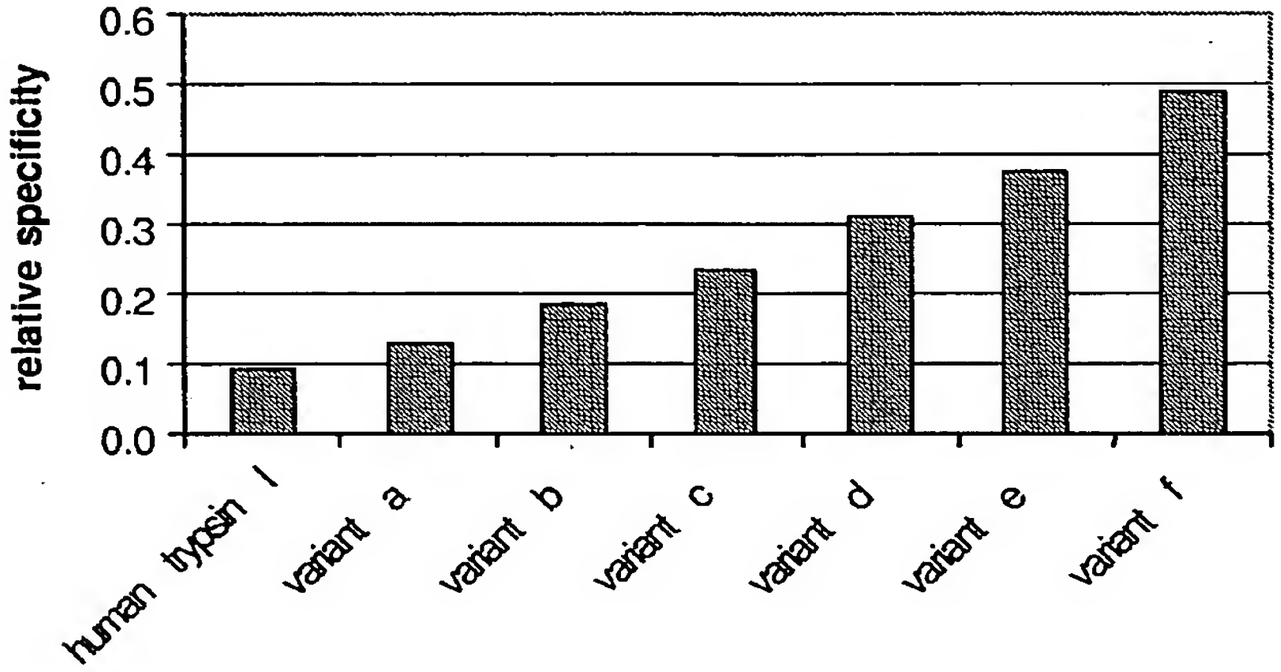
relative specificity
0.0
0.1
0.2
0.3
0.4
0.5
0.6
human trypsin I
variant a
variant b
variant c
variant d
variant e
variant f

Fig. 14

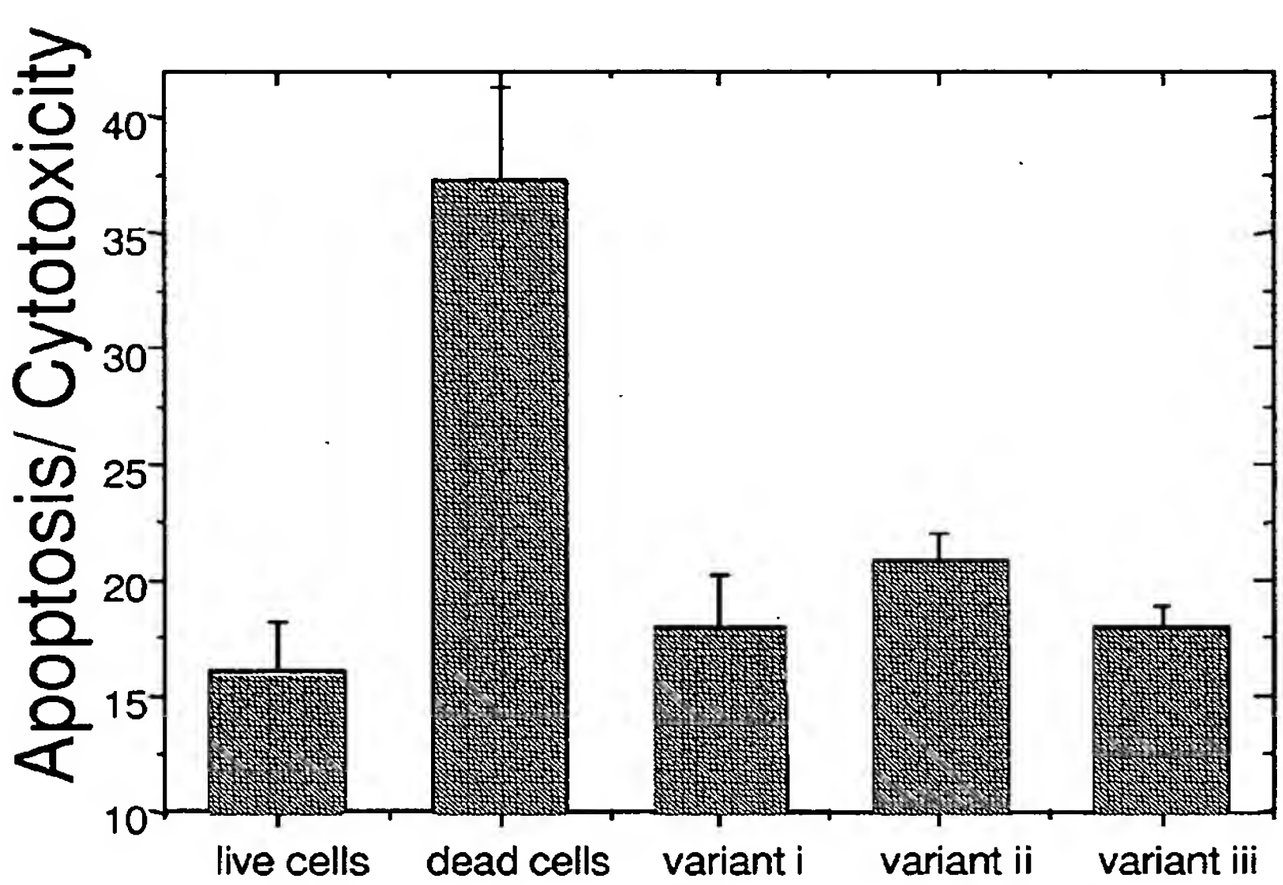
Apoptosis/ Cytotoxicit
40
35
30
25
20
15
10
live cells
dead cells
variant i
variant ii
variant iii

Fig. 15

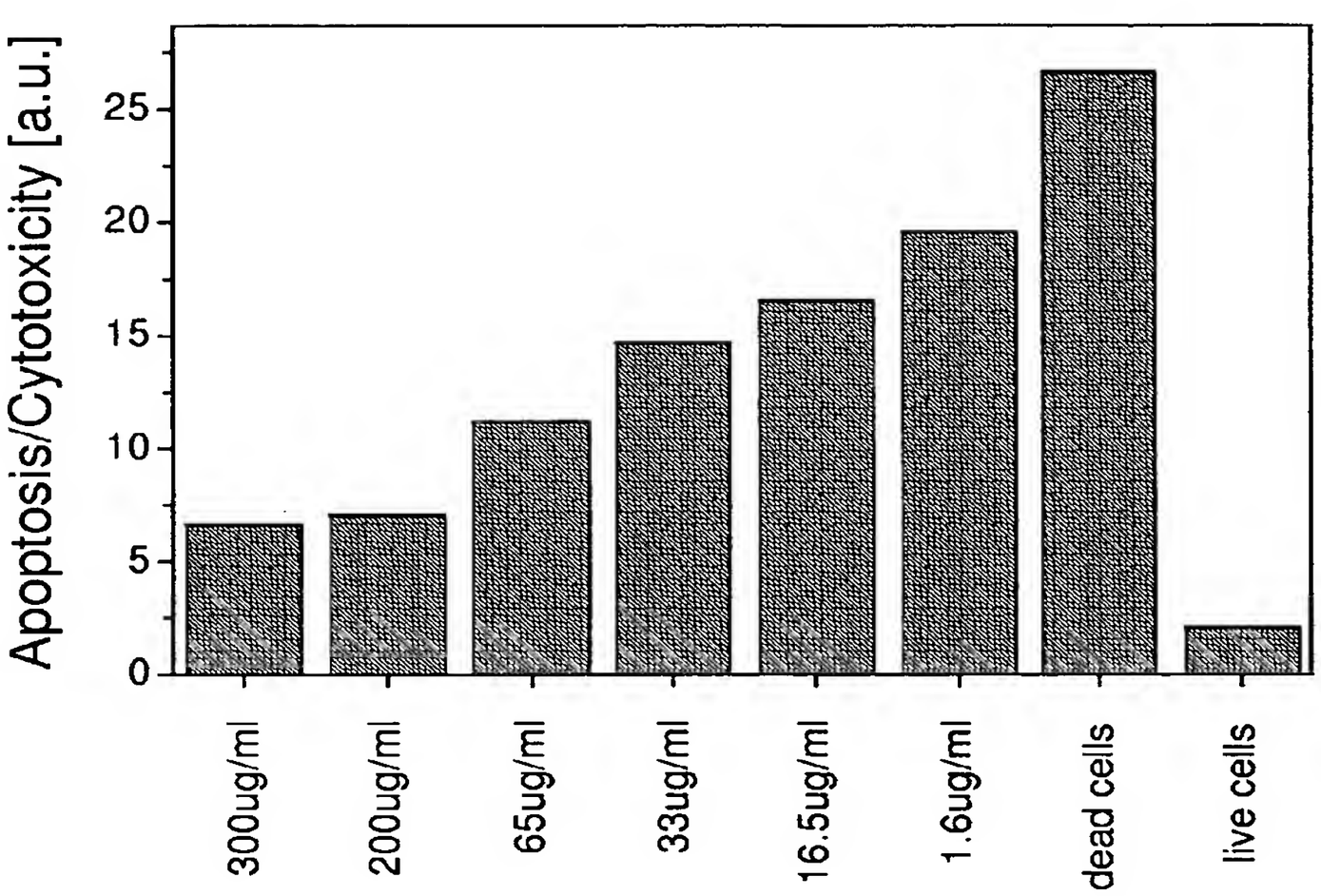
Apoptosis/Cytotoxicity [a.u.]
0
5
10
15
20
25
300ug/ml
200ug/ml
65ug/ml
33ug/ml
16.5ug/ml
1.6ug/ml
dead cells
live cells

Fig. 16

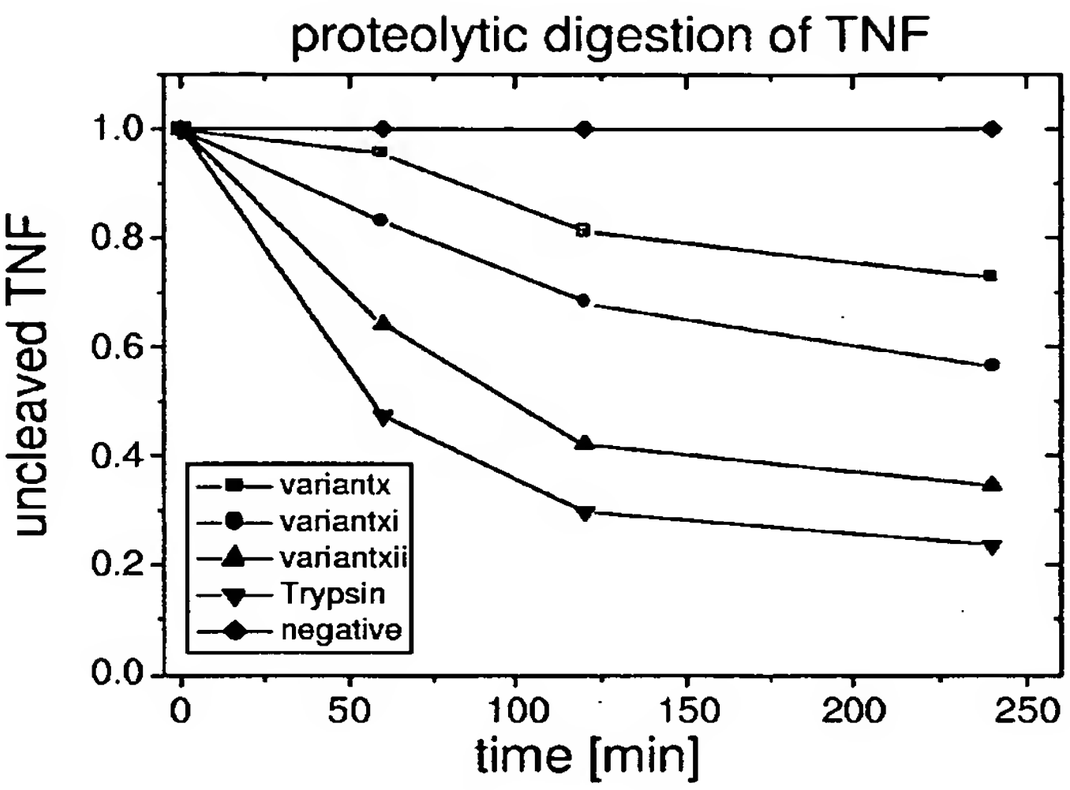

Fig. 17a

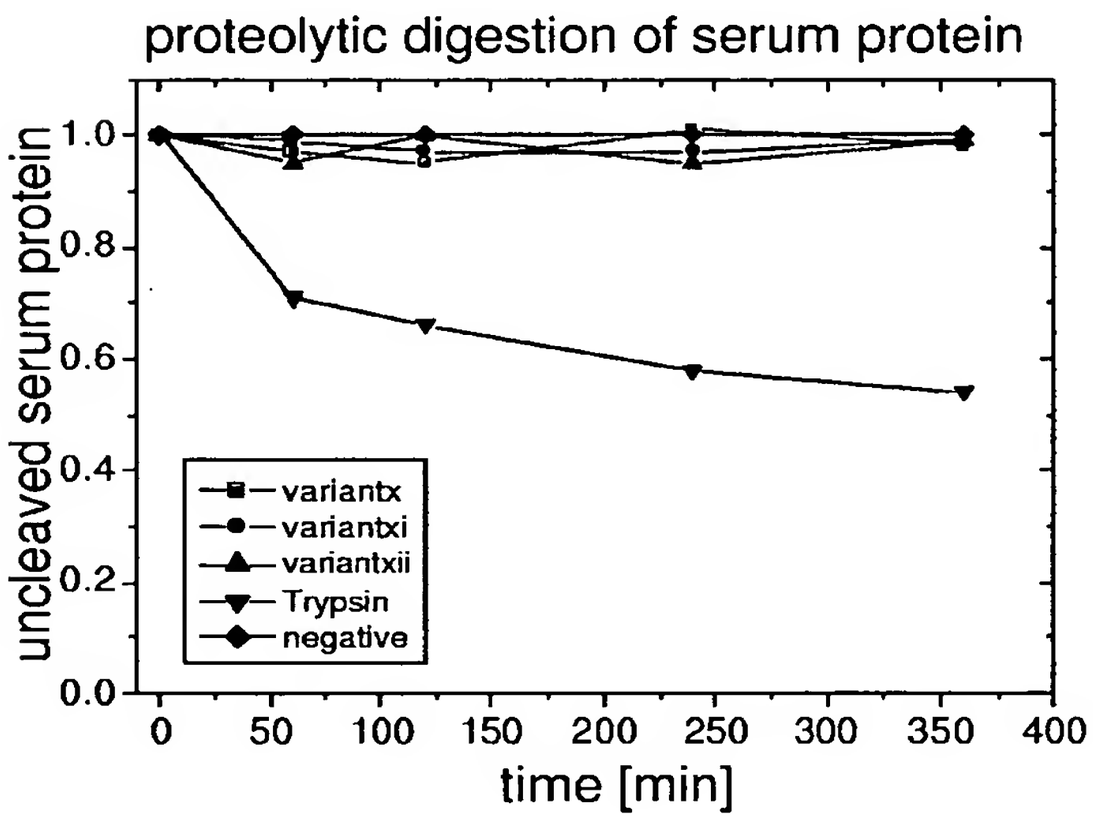

Fig. 17b

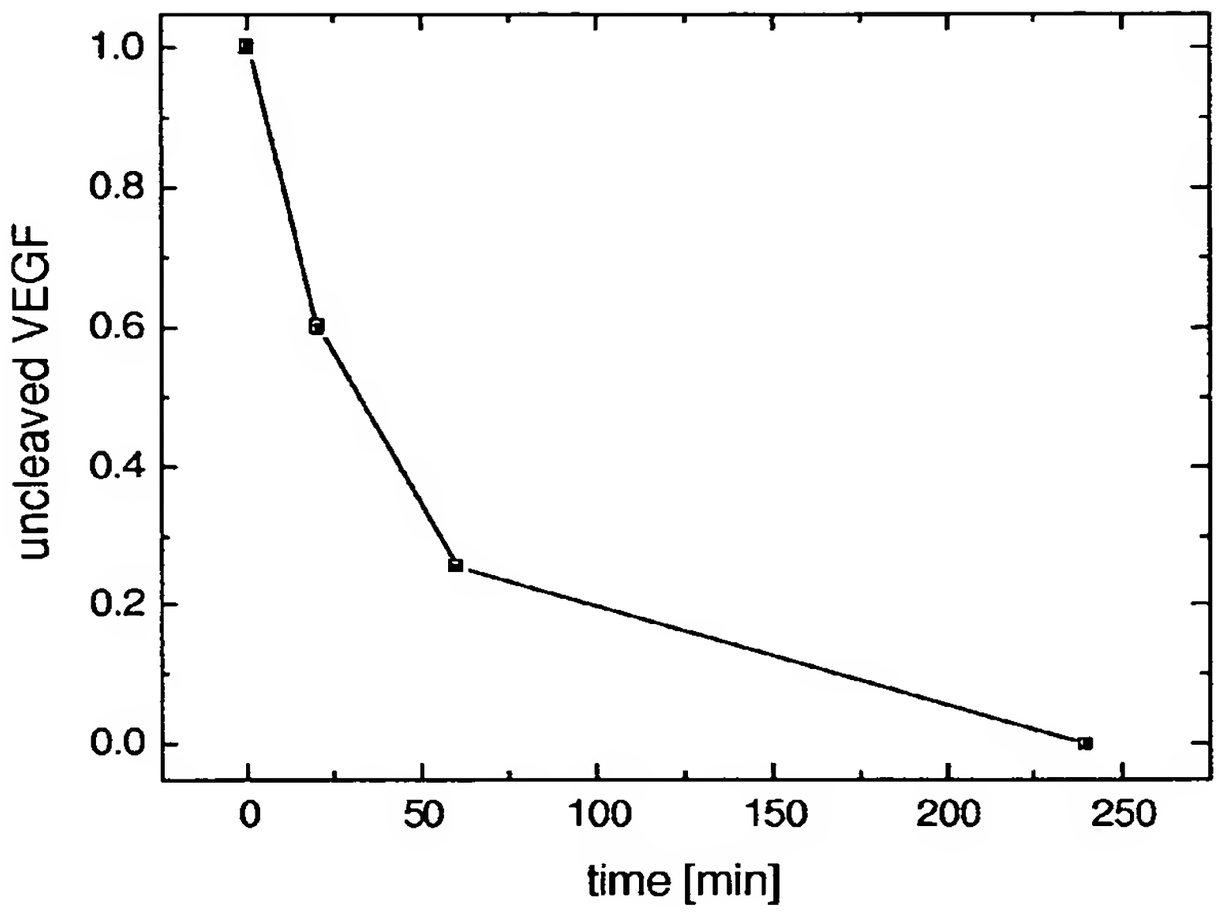

Fig. 18